DATE

The Honorable Gina McCarthy
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Subject: Review of EPA's Draft Assessment entitled *Toxicological Review of Benzo[a]pyrene (September 2014)*

Dear Administrator McCarthy:

The EPA's National Center for Environmental Assessment (NCEA) requested that the Science Advisory Board (SAB) review the draft assessment, entitled *Draft Toxicological Review of Benzo[a]pyrene.* The assessment consists of a review of publicly available scientific literature on the toxicity of benzo[a]pyrene (BaP). The SAB was asked to comment on the scientific soundness of the hazard and dose-response assessment of benzo[a]pyrene-induced cancer and non-cancer health effects. In response to EPA's request, the SAB convened a panel consisting of members of the SAB Chemical Assessment Advisory Committee (CAAC) augmented with subject matter experts to conduct the review. The enclosed report provides the SAB's consensus advice and recommendations. This letter briefly conveys the major findings.

With regard to hazard identification, the SAB agrees that available human, animal, and mechanistic studies support the EPA's conclusions that developmental neurotoxicity, developmental toxicity, male and female reproductive effects, and immunotoxicity are human hazards of BaP exposure. In addition, the SAB agrees with the classification that BaP is *carcinogenic to humans* by all routes of exposure in accordance with EPA's *Guidelines for Carcinogen Risk Assessment*. However, the evidence presented in the assessment does not support EPA's conclusion that forestomach toxicity in rodents, cardiovascular toxicity, and adult nervous system toxicity are not potential human hazards.

For derivation of the oral reference dose (RfD), the EPA has not made a sufficiently strong case that the available developmental endpoints are the most appropriate non-cancer endpoints for deriving an RfD, or that among the available neurodevelopmental endpoints, the most appropriate results have been used. The SAB suggests that the agency give more consideration to the available data on reproductive outcomes including cervical hyperplasia and cervical inflammation, and provide a firmer justification for not selecting these as critical endpoints. The SAB recommends that the EPA consider the overall picture of neurodevelopmental effects from a broader set of the neurodevelopmental endpoints to justify and support the choice of the critical endpoint.

With respect to the application of uncertainty factors (UFs), the SAB recommends that the EPA consider application of $bw^{3/4}$ adjustment for extrapolation from neonatal animal to neonatal human. In addition, EPA should further justify the application of a database uncertainty factor of 3 that is based, in part, on the absence of a multi-generational study or extended one generation study, and the lack of a study

examining functional neurological endpoints following exposure from gestation through lactation.

For derivation of the inhalation reference concentration (RfC), the SAB found that the RfC value provided in the assessment is not scientifically supported. While the endpoint (decreased fetal survival) and key study selected are appropriate, the RfC is based only upon this one study that has some technical deficiencies that decrease the confidence in the resulting data. The rationale for not employing a BMD approach to derive the point of departure is unclear. Regarding UFs, the EPA application of an UF of 3 to address residual uncertainty for interspecies extrapolation is too low, since the particle size used in the study would result in significant deposition in the upper respiratory tract of rodents. Moreover, the effect was found at all exposure levels. So the lowest-observed-adverse-effect level (LOAEL) obtained may not be the "true" LOAEL, and may not be appropriately addressed with the use of an uncertainty factor for extrapolation from a LOAEL to a no-observed-adverse-effect level (NOAEL). The SAB recommends two studies for EPA to consider to develop a more comprehensive dose-response relationship for BaP.

For derivation of the oral slope factor for cancer, the SAB finds that appropriate studies and models were selected for dose-response analysis. However, insufficient justification was provided for derivation of the final slope factor solely based on a single-sex mouse study that produces the largest cancer slope factor. The SAB suggests that data from all studies be incorporated in the derivation of the oral cancer slope factor. The SAB also questions the use of default cross-species scaling applied to all of the tumor sites identified in the two studies. The SAB commented that allometric scaling for alimentary tract sites (larynx, esophagus, forestomach) which can be considered portal-of-entry tumor sites may not be needed.

For derivation of the inhalation unit risk (IUR) for cancer, the SAB finds that EPA has selected an appropriate study for dose-response analysis, and that appropriate models were used to derive the IUR. The SAB recommends additional discussion of key assumptions, conducting sensitivity analyses, and encourages EPA to reconsider the decision not to use epidemiological data to support their derivation of the IUR.

The SAB commends the agency's efforts in deriving the IRIS Program's first dermal slope factor (DSF). However, the proposed DSF is not sufficiently supported scientifically. The SAB recommends that the EPA include two additional studies for review and consider combining results from the mouse skin tumor bioassays to strengthen the derived DSF. The SAB also recommends that the EPA more thoroughly review the evidence of skin cancer in studies of coke, steel and iron, coal gasification and aluminum workers given their relevance for evaluating the appropriateness of using the mouse-based risk assessment model for predicting skin cancer risk in humans.

The assessment used mass rather than mass/area as the dose metric for cancer risk at "low dose" of BaP. The SAB does not have a specific recommendation as to the dose metric, but strongly recommends that in the absence of empirical data, the decision be based upon a clearly articulated, logical, scientific structure that includes what is known about the dermal absorption of BaP under both conditions of the bioassays and anticipated human exposure, as well as the mechanism of skin carcinogenesis of BaP. The SAB also recommends that cancer risk calculated from the derived DSF should use absorbed dose, and not applied dose. Moreover, the SAB recommends that the EPA describe what constitutes a "low dose" when using the mass of BaP as the dose metric.

The SAB believes the chosen cross-species scaling approach should be supported by a coherent logical structure. In addition, differences between mouse and human skin should be considered, such as thickness of and metabolic rates in the target tissue (i.e., the viable epidermis layer).

Finally, the SAB concludes that the available mechanistic studies in humans and animals support a mutagenic mode of action for BaP-induced cancers, and the proposed use of age-dependent adjustment factors is justified.

The SAB appreciates this opportunity to review EPA's *Draft Toxicological Review of Benzo[a]pyrene* and looks forward to the EPA's response to these recommendations.

Sincerely,

Enclosure

NOTICE

This report has been written as part of the activities of the EPA Science Advisory Board, a public advisory committee providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The Board is structured to provide balanced, expert assessment of scientific matters related to problems facing the Agency. This report has not been reviewed for approval by the Agency and, hence, the contents of this report do not represent the views and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use. Reports of the EPA Science Advisory Board are posted on the EPA website at http://www.epa.gov/sab.

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Abbreviations and Acronyms

AhR	aryl hydrocarbon receptor
AIC	Akaike Information Criteria
ADAF	age-dependent adjustment factor
ADHD	attention deficit hyperactivity disorder
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate
ANOVA	analysis of variance
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	benchmark concentration
BMCL	lower 95% confidence limit of the benchmark concentration
BMD	benchmark dose
BMDL	lower 95% confidence limit of the benchmark dose
BMR	benchmark response
BW	body weight
CAAC	Chemical Assessment Advisory Committee
CI	confidence interval
EPA	Environmental Protection Agency
HED	human equivalent dose
HERO	Health and Environmental Research Online
HPBMC	human peripheral blood mononuclear cell
5-HT	5-hydroxytrytamine
IARC	International Agency for Research on Cancer
Ig	immunoglobulin
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	Lowest-Observed-Adverse-Effect Level
MOA	mode of action
NAS	National Academy of Sciences
NCI	National Cancer Institute
NIOSH	National Institute for Occupational Safety and Health
NMDA	N-methyl-D-aspartate
NOAEL	No-Observed-Adverse-Effect Level
NRC	National Research Council
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
ORD	Office of Research and Development
PAH	polycyclic aromatic hydrocarbons
PFC	plague forming cell
PHA	phytohemagglutinin
POD	point of departure
RfC	reference concentration
RDDR	regional deposited dose ratio
ROS	reactive oxygen species
RR	relative risk

TDAR	T-dependent antibody response
UCL	Upper Confidence Limit
UF	uncertainty factor
UF_D	Database uncertainty factor
UF_H	Human inter-individual variability uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WHO	World Health Organization

1. EXECUTIVE SUMMARY

The Science Advisory Board (SAB) was asked by the EPA Integrated Risk Information System (IRIS) program to review the agency's *Draft IRIS Toxicological Review of Benzo[a]pyrene (September 2014)* (hereafter referred to as the assessment). EPA's IRIS is a human health assessment program that evaluates information on health effects that may result from exposure to environmental contaminants. The assessment consists of a review of publicly available scientific literature on benzo[a]pyrene (BaP). The assessment was revised in September 2014 and a summary of EPA's disposition of the public comments received on an earlier draft of the assessment was added in Appendix G of the Supplemental Information to the Toxicological Review.

EPA asked the SAB to conduct a review of the scientific soundness of the conclusions presented in the draft BaP assessment. The SAB panel charged with conducting the review included members of the SAB Chemical Assessment Advisory Committee augmented with additional subject matter experts. An overview of the SAB's recommendations and advice on how to improve the clarity and strengthen the scientific basis of the assessment are presented below and discussed in greater depth in the body of the report.

Literature Search Strategy/Study Selection and Evaluation

In general, the literature search process is well described and documented. While the EPA did a thorough job documenting search terms used to identify studies for evaluation, the SAB notes that search terms for certain potential target organs are included but not others. The SAB recommends that the EPA review the references in the primary and secondary literature to identify potentially relevant articles not identified through the systematic searching and manual screening processes. In addition, secondary literature searches should be conducted whenever evidence for additional effects (e.g., cardio) and specific data gaps emerge.

The SAB appreciates that EPA is developing a handbook which will outline the tools and processes to address study quality and risk of bias. In the interim, the EPA should provide sufficiently detailed criteria for each step of the process leading to the selection of key studies for the establishment of a point of departure. This will ensure not only that the rationale for initial study inclusion or exclusion are understood, but also that the strengths and weakness of the evaluated studies will be fully transparent.

The SAB found that requiring a direct measure of BaP exposure to be unnecessarily restrictive, especially when evaluating epidemiology studies, as these studies would be relevant for hazard identification. Epidemiological studies of coke oven workers and other occupational groups with known exposures to BaP should at least be reviewed in the tables if not the text. The review of the epidemiology studies presented in the supplemental information relied heavily on the systematic review and meta-analysis reported by Bosetti et al. (2007) and Armstrong et al. (2004), respectively. It seems inappropriate for EPA to rely solely on review articles rather than a review of the primary literature. In addition, the draft Supplemental Information document does not discuss any of the studies of asphalt workers and roofers or coke oven workers. Some of the studies of coal tar that were identified in the public comments were not included in the EPA review.

The SAB has provided a list of peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene.

Hazard Identification

Developmental Neurotoxicity and Developmental Toxicity

The SAB concurs with EPA that BaP is a developmental neurotoxic agent in animals with supporting evidence in humans. Prenatal and early life airborne PAH exposures have been found to affect children's IQ adversely and may also contribute to ADHD behavior. In addition, there were plausible mechanistic studies that implicate NMDA and AMPA glutamate receptors, as well as 5-HT receptors, as potentially mediating the observed neurobehavioral effects. Thus, there are sufficient studies, when considering the human, animal and mechanistic studies, to provide enough evidence of developmental neurotoxicity and effects on brain development and behavior. While each study has limitations, the weight of evidence supports BaP as developmentally neurotoxic.

The SAB concurs with the EPA that the available human studies support a contribution of BaP to human developmental toxicity. Studies with PAH mixtures have shown a correlation between PAH exposure and lower birth weights, increased risk of fetal death, and BaP DNA adducts. BaP exposure *in utero* has been demonstrated to cause fetal death, lower fetal/offspring weights and affect fetal germ cells. Additional studies that should be considered for inclusion include reported BaP-related effects on fetal lung growth/function, and teratogenicity.

Reproductive Toxicity

The SAB agrees that the data support the conclusion that BaP is a male and female reproductive toxicant through the oral and inhalation routes of exposure. The rodent data demonstrate convincingly that BaP affects fertility and fecundity. The functional effects in male rodents include adverse changes in testes and sperm and hormonal changes. Similar changes in sperm quality and fertility have been detected in humans exposed to PAH mixtures. The SAB recommends that EPA give greater consideration to the genotoxic effects of BaP on male germ cells as a possible mode of action. BaP is mutagenic and mutagenesis in the germline can be detrimental to reproductive health.

BaP has a direct effect on adult rodent ovarian follicles. A recent study showed that *in vivo* exposure to BaP induces significant DNA damage in mouse oocytes and cumulus cells. *In utero* exposure of developing females to BaP provides compelling evidence that there is a sensitive window for exposure to BaP for the developing ovary.

Immunotoxicity

The SAB finds that the available immunotoxicity data based on animal models of pure BaP and complex PAH mixture exposures to humans (coke oven workers) support the claim that BaP is a human hazard for the immune system. The evidence for immunotoxicity in humans is based upon complex PAH mixture exposures. BAP as a pure chemical can cause suppression of human peripheral blood mononuclear cell responses at low concentrations (10-100nm) *in vitro*. Immunotoxicity is caused by a combination of genotoxic (DNA adducts and p53-induced cell death) and non-genotoxic mechanisms (signaling due to AhR activation and oxidative stress). Animal studies provide strong evidence that BaP suppresses immune function leading to adverse consequences for host resistance to infections. In addition to the evidence that BaP alters T cell development *in utero* and in adults, there is evidence that

BaP alters B cell development in the bone marrow of adults. It is likely that the developing immune system may be one to two orders of magnitude more sensitive to BaP exposures than adult exposures.

Cancer

The SAB finds that, in accordance with EPA's Cancer Guidelines (USEPA, 2005a), the EPA has demonstrated that benzo[a]pyrene is a human carcinogen. This conclusion was based primarily on: (1) extensive evidence of carcinogenicity in animal studies, (2) the mode of carcinogenic action – mutagenic, and associated key precursor events have been identified in animals, (3) strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, and (4) strong support from an excess of lung cancer in humans who were exposed to PAHs, although not to benzo[a]pyrene alone. This conclusion is consistent with the evaluations by other agencies, including the World Health Organization International Agency for Research on Cancer (2010) and Health Canada (2015).

Other Toxicity

The potential hazards from BaP exposure identified and discussed in Section 1.1.4 include forestomach toxicity, hematological toxicity, liver toxicity, kidney toxicity, cardiovascular toxicity, and adult nervous system effects. Overall, the EPA concluded that the available evidence does not support these noncancer effects as potential human hazards. The SAB recommends that EPA's basis for arriving at this conclusion be expanded for these health endpoints. In addition, the SAB finds that the evidence presented in the assessment does not support EPA's conclusion that forestomach toxicity in rodents, cardiovascular toxicity, and adult nervous system toxicity are not potential human hazards. The SAB also notes that the literature search was not sufficiently comprehensive to identify studies relevant to the characterizaton of cardiovascular system toxicity due to BaP exposure. Furthermore, the SAB identifies adult and developmental pulmonary toxicity as noncancer endpoints that can be credibly associated with BaP exposure, but were not identified in the draft assessment.

Dose-Response Analysis

Oral Reference Dose for Effects Other Than Cancer

The SAB agrees that developmental endpoints, and in particular, neurodevelopmental endpoints are, an appropriate basis for deriving an RfD for BaP. However, the SAB does not find that EPA has made a sufficiently strong case that the available developmental endpoints are the most appropriate non-cancer endpoints for setting an RfD, or that among the available neurodevelopmental endpoints, the observed results from the elevated plus maze test in Chen et al. (2012) are the most appropriate results.

With respect to developmental toxicity as the most appropriate category of non-cancer effects, the SAB suggests that EPA give more consideration to the available reproductive outcomes including cervical hyperplasia and cervical inflammation in Gao et al. (2011), and at least provide a firmer justification for not selecting these as critical endpoints.

With respect to the choice of specific neurodevelopmental endpoints, the SAB recommends that the EPA consider the overall picture of neurodevelopmental impact from all of the neurodevelopmental endpoints in Chen et al. (2012)—including plus maze, reflex, locomotor activity and water maze—to justify and support the choice of the critical endpoint. In particular, the SAB suggests that EPA reconsider or provide stronger justification for not using escape latency from the Morris water maze.

With respect to the application of uncertainty factors, the SAB recommends that the EPA consider application of $bw^{3/4}$ adjustment as per the agency's 2011 allometric scaling guidance for extrapolation from neonatal animal to neonatal human. In addition, the SAB recommends that EPA further justify the application of a database uncertainty factor of 3 that is based, in part, on the absence of a multi-generational study or extended one generation study, and the lack of a study examining functional neurological endpoints following exposure from gestation through lactation. The EPA might consider whether an EPA developmental neurotoxicity guideline study and/or extended 1-gen study with a DNT cohort is likely to result in a NOAEL below that of Chen et al. (2012).

Inhalation Reference Concentration for Effects other than Cancer

The RfC value as provided in the draft assessment is not currently scientifically supportable due to: (1) the use of only one study (Archibong et al., 2002) for determining the POD, (2) some design and technical issues with this study, and (3) issues involving UF values. The rationale for not employing a BMD approach is unclear. Regarding uncertainty factors, given the particle size used in the study would result in significant deposition in the upper respiratory tract of rodents, and the regional deposited dose ratio (RDDR) adjustment used with the key study does not account for systemic toxicokinetics. The EPA application of a UF of 3 to address residual uncertainty for interspecies extrapolation is too low. Moreover, the Archibong et al. (2002) study found effects at all exposure levels. Thus, the LOAEL obtained may not be the "true" LOAEL for this endpoint (decreased fetal survival) and may not be appropriately addressed with the use of an uncertainty factor of 10 for extrapolation from LOAEL to NOAEL. The SAB recommends that EPA consider studies by Wu et al. (2003) and Archibong et al (2012). While these two studies are not replicates of the key study, they may be useful in developing a more comprehensive dose-response relationship for BaP and, thus, perhaps increasing confidence in the LOAEL value used.

Oral Slope Factor for Cancer

The SAB finds that appropriate studies and models were selected for dose-response analysis. However, an insufficient justification was provided for the selection of the final slope factor solely from the Beland and Culp (1998) mouse study, instead of the slope factor from the Kroese et al. (2001) rat study, or an average of the two, i.e., EPA's choice of the single-sex mouse study that produces the largest cancer slope factor instead of slope factor that incorporates data from all studies. The SAB also has questions regarding the choice of cross-species scaling factors. Using this approach, time-weighted daily average doses are converted to human equivalent doses (HEDs) on the basis of $bw^{3/4}$ scaling. This allometric scaling is based on current EPA guidelines. However, there is uncertainty as to whether this scaling should be applied to all of the tumor sites identified in the two studies. In particular, alimentary tract sites (larynx, esophagus, forestomach) can be considered portal-of-entry tumor sites, and allometric scaling may not be appropriate for these sites.

Inhalation Unit Risk for Cancer

The SAB concludes that EPA has selected an appropriate study (Thyssen et al., 1981) for dose-response analysis and that appropriate models were used to derive the inhalation unit risk (IUR). Although the IUR value is scientifically supported, the SAB recommends additional discussion of the key assumptions, conducting several sensitivity analyses, and reconsideration of the use of epidemiological data to derive inhalation unit risk values. The SAB also suggests the inclusion of an explicit conclusion statement regarding overall uncertainty of the unit risk value, and a brief discussion of the applicability of this value to typical environmental exposures (especially for sensitive subpopulations).

Dermal Slope Factor for Cancer

The SAB found the proposed dermal slope factor (DSF) and the cross-species scaling to be not sufficiently scientifically supported. The key findings and recommendations of the SAB are summarized below:

- Choice of Studies:

  The draft BaP assessment reviewed 10 complete carcinogenicity mouse skin tumor bioassays and Sivak et al. (1997) was chosen as the principal study. The SAB recommends that EPA consider adding Nesnow et al. (1983) and Levin et al. (1997) for review and consider combining results from the different studies to strengthen the derived DSF. The SAB also found EPA's review of the epidemiological evidence of skin cancer in humans not sufficiently thorough. The SAB recommends that EPA more thoroughly review the evidence for skin cancer in studies of coke, steel and iron, coal gasification and aluminum workers given their relevance for evaluating the appropriateness of using the mouse based risk assessment model for predicting skin cancer risk in humans. The SAB agrees with EPA that epidemiologic studies of therapeutic use of coal tar preparation do not provide an adequate basis for either hazard identification or the derivation of a dermal slope factor.

- Dose-Response Analysis:

  The draft BaP assessment used mass rather than mass/skin area as the dose metric for cancer risk at "low doses" of BaP. Published dermal slope factors for BaP skin carcinogenesis have used mass and mass/skin area as dose metrics and there does not appear to be any empirical data available to inform a choice between these two dose metrics or another metric. The SAB does not have a specific recommendation as to BaP dose metric, but strongly recommends that in the absence of empirical data, the decision be based upon a clearly articulated, logical, scientific structure that includes what is known about the dermal absorption of BaP under both conditions of the bioassays and anticipated human exposures, as well as the mechanism of skin carcinogenesis of BaP. The SAB recommends that cancer risk calculated from the derived DSF should use absorbed dose, and not applied dose. The SAB also recommends that the EPA describe what constitutes a "low dose" if the assumption that mass of BaP is the appropriate dose metric for calculating the DSF from the skin cancer bioassay and for estimating cancer risk in humans.

- Dermal Slope Factor Cross-Species Scaling:

  Experimental cancer risk information for scaling from mouse to human skin cancer resulting from dermal exposure is not available. The science for selecting the allometric scaling approach employed by EPA using body weight to the ¾ power is uncertain. However, the chosen cross-species scaling approach should be supported by a coherent logical structure. In addition, differences between mouse and human skin should be considered, such as thickness of and metabolic rates in the target tissue (i.e., the viable epidermis layer).

The SAB has made other recommendations for describing the cancer risk calculated with the DSF. Some of the recommendations include the need for EPA to calculate the cancer risk from the absorbed dose, and state clearly how the absorbed dose is estimated from the exposed dose. In actual BaP exposures (from soil and other environmental media), the absorbed dose should be estimated from the exposed dose and the exposure scenario.

Age-dependent Adjustment Factors for Cancer

The SAB finds that the available mechanistic studies in humans and animals support a mutagenic mode of action for BaP-induced cancers. Given that the EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposures to Carcinogens* establishes a rational approach for the adjustment of tumor risk for exposures at different ages for carcinogens with a mutagenic mode of action, the SAB concludes that the proposed use of age-dependent adjustment factors (ADAFs) is justified.

Executive Summary

The SAB found that the major conclusions of the EPA draft assessment for BaP were clearly and appropriately presented in the Executive Summary. Changes made to the body of the assessment in response to the SAB recommendations regarding the derivation of the chronic RfD/RfC, or cancer slope factors, should be incorporated into the Executive Summary. In addition, the SAB provides a number of suggestions for improvement of the Executive Summary.

Disposition of Public Comments

The SAB found that most of the scientific issues raised by the public, as described in Appendix G, were adequately addressed by the EPA. However, there were some issues on which the SAB differs from the EPA responses or provides additional comments on the topic. These issues were identified and referenced to relevant sections of the SAB report.

2. INTRODUCTION

The Science Advisory Board (SAB) was asked by the EPA Integrated Risk Information System (IRIS) program to review the agency's *Draft IRIS Toxicological Review of Benzo[a]pyrene* (hereafter referred to as the assessment). EPA's IRIS is a human health assessment program that evaluates information on health effects that may result from exposure to environmental contaminants. The assessment consists of a review of publicly available scientific literature on benzo[a]pyrene (BaP). The assessment was revised in September 2014 and a summary of EPA's disposition of the public comments received on an earlier draft of the assessment was added in Appendix G of the Supplemental Information to the Toxicological Review.

In response to the agency's request, the SAB convened an expert panel consisting of members of the Chemical Assessment Advisory Committee augmented with subject matter experts to conduct the review. The SAB panel held a teleconference on March 4, 2015 to discuss EPA's charge questions (see Appendix A), and a face-to-face meeting on April 15-17, 2015 to discuss responses to charge questions and consider public comments. The SAB panel also held teleconferences to discuss their draft reports on August 21, 2015 and September 2, 2015. Oral and written public comments have been considered throughout the advisory process.

This report is organized to follow the order of the charge questions. The full charge to the SAB is provided as Appendix A. Additional peer-reviewed studies on health effects of BaP are provided in Appendix B. Suggestions on the format for EPA's charge questions are provided in Appendix C.

3. RESPONSES TO EPA'S CHARGE QUESTIONS

3.1. Literature Search/Study Selection and Evaluation

Charge Question 1. The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the Literature Search Strategy/Study Selection and Evaluation section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene

The literature review process is well described and documented. The EPA did a thorough job documenting search terms used to identify studies in the main and supplementary report. In reviewing the initial literature search strategy keywords (Table LS-1 and Appendix C), the SAB noted that search terms for certain potential target organs are included but not others. To ensure that the literature search was comprehensive and bias was avoided, the SAB recommends that EPA specify whether the search strategy included: (1) a review of the references in the primary and secondary literature as a means to identify potentially relevant articles not identified through the systematic searching and manual screening processes, and (2) conducting secondary literature searches as evidence for additional effects (e.g., cardio) or specific data gaps (e.g., MOA, *in vitro* studies) emerged. These steps should explicitly be included in the literature search and study selection strategy.

Figure LS-1 is helpful in identifying the general criteria used for study selection/exclusion. However it is difficult to assess what information has been lost due to the exclusion of ~600 articles originally retrieved using the search criteria (3rd dotted line box) and why. It is appropriate to exclude papers that are "not relevant to BaP toxicity in mammals," or have "inadequate reporting of study methods or results" or "inadequate basis to infer exposure." The SAB appreciates that EPA is developing a handbook which will outline the tools and processes to address study quality and risk of bias. In the interim EPA should provide sufficiently detailed criteria for each step of the process leading to the selection of key studies for the point of departure (POD) assessment. This will ensure that not only the rationale for initial study inclusion or exclusion are clearly understood, but also that the strengths and weaknesses of studies selected (as well as those that are not) for POD assessment are fully transparent. EPA may want to consider identifying these criteria in one location within the Literature Search and Study Selection section, rather than directing the reader to other sections of the document or EPA references.

To increase transparency regarding excluded studies the SAB recommends that a table containing the list of excluded references, grouped by the applicable exclusion criteria, be included in the supplementary information. For the BaP assessment this will provide needed clarity regarding which epidemiological studies and animal studies were eliminated due to inadequate basis to infer exposure, inadequate reporting of study methods/results, and studies with mixtures.

The assessment separated the identified epidemiologic studies into tiers according to the extent and quality of the exposure analysis and other study design features. Tier 1 studies have detailed exposure

assessment, large sample size, and adequate follow-up period. Tier 2 studies did not meet the criteria for Tier 1 regarding exposure assessment, sample size, or follow-up period. SAB finds requiring a direct measure of BaP exposure unnecessarily restrictive, especially in regards to epidemiology studies, as these studies would be relevant for hazard identification. Epidemiological studies of coke oven workers and other occupational groups with known exposures to BaP are valuable sources of information for determining causality even if they do not include quantification of BaP exposures. These studies should at least be reviewed in the tables if not the text. The assessment only considered three epidemiology studies met this criterion for Tier 1 for lung cancer (Armstrong and Gibbs 2009; Spinelli et al. 2006; Xu et al. 1996) and bladder cancer (Gibbs and Sevigny 2007a, 2007b; Spinelli et al. 2006; Burstyn et al. 2007). The Tier 1 studies only included studies of the aluminum and iron and steel manufacturing. It did not include any studies of workers from the coke ovens, and roofing or asphalt industries which would have very high exposures to BaP and thus should be relevant for determining causality even though they may not have had detailed exposure assessments for BaP. Tier 2 studies are presented in a table in the assessment. However, there are many studies missing from these tables (e.g., Romunstadt et al. 2000; Ronneberg 1999, that have been included in prior assessments (i.e., see Table 1 in Bosetti et al. 2007 and Rota et al. 2014).

The review of epidemiology studies presented in the supplemental information section relied heavily on a systematic review and meta-analysis reported by Bosetti et al. (2007) and by Armstrong et al. (2004). It seems inappropriate for EPA to rely solely on review articles rather than a review of the primary literature. There is also a more recent meta-analysis that was not included in the assessment (Rota et al. 2014). Many of the epidemiologic studies cited in Bosetti and Rota are not discussed in the EPA Supplemental Information document. For aluminum production workers the EPA only discusses the studies by Spinelli et al. (1991, 2006), Romundstad et al. (2000a, 2000b) and Xu et al. (1996). There are 10 other studies of aluminum production workers cited in the Bosetti review (see Table 1 of Bosetti et al. 2007), and five additional studies cited in the Rota review article (see Table 1 of Rota et al., 2014). It is unclear why the EPA only included the few epidemiologic studies that they did review in their assessment.

For asphalt and roofers, the Supplemental Information document refers the readers to the Bosetti et al. (2007) review. Five papers were cited to provide evidence of an excess risk of lung cancer and weak evidence for bladder cancer among asphalt workers and roofers (Burstyn 2007; Partanen and Bofetta 1994; Chiazze et al. 1991; Hansen 1989, 1991; Hammond et al. 1976). Studies cited in Bosetti (see Table 1) of roofers by Swaen et al. (1991) and of asphalt workers cited in Rota (see Table 1) by Behrens et al. (2009) and Zanardi et al. (2013) seem to have been overlooked. For coke oven workers, coal gasification and iron and steel foundry workers the supplemental document relies entirely on the reviews by Boffetta et al. (1997), Bosetti et al. (2007) and Armstrong et al. (2004). The more recent review by Rota et al. (2014) identified two new studies of iron and steel workers (see Table 1) that were not considered in the earlier reviews.

Finally, it is not clear why some of the studies of coal tar that were identified in the comments from the American Coke and Coal Chemicals Institute were not included in the EPA assessment. In particular the studies by Bhate et al. (1993), Hannuksela-Svahn et al. (2000), Jemec and Østerlind (1994), Jones et al. (1985), Menter Cram (1983), and Muller and Kierland (1964) seem to meet the criteria for review, although the SAB noted that limitations in these studies make them of limited value for the assessment.

It also appears that *in vitro* studies (other than genotoxicity studies) and animal *in vivo* studies designed to identify potential therapeutic agents that would prevent the carcinogenicity or genotoxicity of BaP were not included. It would be expected that such studies might provide additional information on mode of action of BaP.

In Appendix B, the SAB recommends a number of additional peer-reviewed studies from the primary literature, including some that are in HERO but were not used in the assessment, that the agency should consider in the assessment of noncancer and cancer health effects of BaP.

3.2. Hazard identification.

In section 1 of the draft assessment, the EPA evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with BaP exposure. The draft assessment uses EPA's guidance documents to reach conclusions about developmental toxicity, reproductive toxicity, immunotoxicity, carcinogenicity and other types of toxicity associated with BaP exposure. The SAB discusses the strength of the scientific evidence for each of these types of toxicity in the sections that follow.

3.2.1. Developmental toxicity

Charge Question 2a. The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human and animal studies support this conclusion?

The SAB considered sudivided this Charge Question in two parts: developmental neurotoxicity; and developmental toxicity other than neurodevelopment.

Developmental Neurotoxicity

The SAB found the assessment to be thorough with regard to identifying studies pertaining to developmental neurotoxicity and found no additional literature. The SAB concurs with the EPA that the available human studies support the conclusion that BaP exposure contributes to human developmental neurotoxicity. There are relevant human epidemiological studies on developmental effects on neurodevelopment resulting from exposure to BaP-PAH mixtures (Perera et al. 2004, 2005, 2009, 2011, 2012a; 2012b; Tang et al. 2006, 2008). For example, in a prospective cohort study in New York City, prenatal exposure to airborne PAH was found to affect children's IQ adversely (Perera et al. 2009). When the cohort was followed to the age of 9 years, the investigators concluded that early life exposure to environmental PAH may also contribute to attention deficit hyperactivity disorder (ADHD) behavior problems in children (Perera et al. 2014). The EPA assessment appropriately notes that in human studies the exposures are to PAH mixtures, and, therefore, the effects of BaP alone on child neurodevelopment cannot be isolated and determined to be exclusively attributable to BaP rather than the sum, interaction, or antagonist effect of multiple PAHs acting in concert. However, the human prospective cohort studies have many strengths. These include the fact that, (1) they are conducted in the target species (human), (2) they are prospective, and (3) they are from two separate populations with one cohort followed from before birth to the age of 9 years. An important aspect of the human studies that add additional weight to their validity is that they measured BaP-specific DNA adducts in maternal and umbilical plasma and also used individually worn air samplers on the mothers and found general agreement between the air sampling and internal dose metrics. Of importance is that the method used for the BaP DNA adduct

determinations was specific for BaP adducts and not generic for other PAH DNA adducts. The fact that the New York City Children Study (Perera et al. 2014) used a specific DNA adduct assay for BaP (Alexandrov et al. 1992) is a significant strength of these data.

The SAB also concurs with the EPA assessment that the animal data support the view that BaP is developmentally neurotoxic in rodents. The SAB concludes that the assessment correctly identified the key studies, but did not consistently address the quality of the studies. Of these the Chen et al. (2012) study was viewed as providing the best evidence despite some deficiencies. This study has a number of strengths; these included (1) using in-house breeding, (2) using 40 litters, (3) standardizing litter size, (4) blind observations of subjective behaviors, (5) balancing the time of testing across dose group, (6) testing multiple dose levels of BaP, (7) administering BaP by gavage, (8) efforts to neutralize litter effects, (9) use of multiple behavioral tests, (10) appropriate ANOVA methods as the main way of analyzing the data (see caveat below on post hoc testing), and (11) use of the Morris water maze (MWM). The study used a split-litter design which has both strength and weakness (discussed at the end of next paragraph).

The SAB has also identified weaknesses in Chen et al. (2012). The MWM was undersized for adult rats, reliance on latency as the sole index of performance on learning trials may be insufficient without swim speed data; however, they report no swim speed differences on the probe trials. The use of the Least Significant Difference (LSD) test is a concern as it over-emphasizes differences as significant that may not be. The EPA assessment correctly notes the importance of the parallelism of the learning curves. Learning rate was not shown to differ between groups. Rather the significant differences in latency between treatment groups seen throughout testing was likely due to some other long-lasting behavioral effect caused by developmental BaP exposure. The EPA also expressed concern about the interpretative value of the probe trial data in light of the fact that the affected BaP groups never reached the same level of proficiency on the learning trials as controls prior to being tested for memory and this concern remains. The pup randomization and litter rotation procedure used in the study is an unproven method of trying to prevent litter effects. It may work as intended or it may introduce unknown effects. While effects, if any, would be expected to be randomly distributed across litters, there exists the potential for interactions between groups created by this method of transferring pups between dams. Concern was raised about having all dose groups within litters. This could cause cross contamination of BaP from higher dose groups to lower dose or control groups. Further, it is unknown if the dams could distinguish differences among the differently dosed pups and thereby differentially care for her offspring.

Despite these concerns and despite issues concerning whether the data reflect a spatial learning deficit or not, the MWM data show a BaP dose-dependent effect. Compared to the Elevated Plus Maze (EPM) data, the increased escape latency in the MWM appears to be a more stable behavioral change that was repeated over 4 days for two separate tracks (cohorts) of animals. Rather than placing reliance only on the EPM data and dismissing the Morris water maze data, the SAB recommends taking into account all the data in this study collectively and viewing them in their totality as evidence of a developmental neurobehavioral effect of neonatal BaP exposure with long-term adverse central nervous system effects.

The SAB understands the EPA's desire to use the Chen et al. (2012) data to generate an RfD. Given the uncertainties identified, however, the assessment should consider if the resultant RfD emphasizing the EPM effects is the most appropriate outcome, or using other end points, including the MWM results, may be more stable and reliable.

The SAB further notes that the Chen et al. (2012) data are supported by other studies. Bouayed et al. (2009) used mice treated with 0, 2 or 20 mg/kg BaP by gavage on postnatal day 0-14, assessed at different ages, and appropriate statistical analyses were used. This is a low-quality study with inadequate (small) sample size of five litters/dose, oversampling of four pups/litter without including litter as a factor in the statistical analyses, and no mention of whether the observations were conducted blind to treatment level and the order of testing counterbalanced across treatment level. Nevertheless, many of the tests were affected and the data were generally in alignment with those of Chen et al. (2012).

Tang et al. (2011) treated Wistar rats starting at weaning for 14 weeks with 1, 2.5, or 6.25 mg/kg BaP i.p. from postnatal day 21 onward. Although the route of exposure is not directly relevant to humans, they too found increases in MWM latency as their measure of learning and on the probe trial to test for reference memory. They found effects at all doses of BaP. The study had reasonable group sizes (9/group), reasonable learning curves, and the data were appropriately analyzed. They too relied on latency as their index of learning but their findings are in general agreement with those of Chen et al. (2012).

Relevant to the derivation of the inhalation RfC, the Wormley et al. (2004) paper is an inhalation nose-only developmental neurotoxicity study. The restraint required in a nose-only study can induce stress in the dams, which can cause long-lasting neurobehavioral effects in the offspring (Markham et al. 2010).

The SAB concurs with the EPA that there were plausible mechanistic studies identified for how BaP may affect neurobehavioral development. Brown et al. (2007) and McCallister et al. (2008) treated rats with BaP by oral gavage on gestational days 14-17 and found metabolites in higher concentrations in brain than liver of the offspring. In addition, *in utero* BaP exposure reduced mRNA expression of glutamate receptor subunits, NMDA-NR2A and NR2B, and AMPA receptor expression and protein concentrations in hippocampus and inhibited NMDA-dependent cortical barrel field post-stimulation spikes by 50 percent. Bouayed et al. (2009) gave Swiss mice BaP on PND 0-14 and found effects on surface righting, forelimb grip, and EPM similar to that found by Chen et al., along with reduced spontaneous alternation and brain mRNA expression of 5-HT1A receptor. These findings implicate NMDA and AMPA glutamate receptors, as well as 5-HT receptors as potentially mediating the neurobehavioral effects seen by Chen et al. (2012) and others. They also support the view that developmental exposure to BaP adversely effects brain development and behavior.

The SAB concluded that the EPA correctly identified BaP as a developmental neurotoxic agent in animals with supporting evidence in humans. There are sufficient studies that when reading across the human, animal, and mechanistic data, they provide evidence of developmental neurotoxicity and that the data are convergent in showing BaP effects on brain development and behavior. While each study has limitations, the weight of evidence supports BaP as developmentally neurotoxic.

Developmental Toxicity

The SAB concurs with the EPA that the available human studies also support a contribution of BaP to human developmental toxicity. Studies with PAH mixtures have shown a relationship amongst PAH exposure, lower birth weights, increased risk of fetal death, and BaP DNA adducts formation (see also Dejmek et al. 2000).

The SAB also concurs with the EPA that the available animal studies support the conclusion that BaP is a developmental toxicant in animals. BaP exposure *in utero* has been demonstrated to cause fetal death, lower fetal/offspring weights and to affect fetal germ cells. Additional studies that should be considered include reports on BaP-related effects on fetal lung growth/function (Thakur et al., 2014) and teratogenicity (Shum et al., 1979; Rigdon and Rennels 1964; Nebert et al, 1977).

A brief survey of the literature indicates that there are additional reports that provide perspective on the likely mode/mechanism of action leading to BaP-related developmental toxicity that are not mentioned in the draft document. For example, there are studies on the formation of BaP adducts in rapidly dividing cells, including fetal tissues (Lu et al., 1986), the severity of developmental toxicity associated with Ah receptor status (Nebert et al., 1977), and the role of oxidative stress (Wells et al. 1997; Nakamura et al. 2012; Thakur et al. 2014). Therefore, the SAB suggests that EPA consider including additional examples, as warranted, of mechanistic studies.

Toxicokinetic information regarding fetal exposures (Shendrikova and Aleksandrov, 1974; Schlede and Merker 1972) and lactational transfer should also be included as they inform the comparative doses to developing organisms at different stages of development and exposed via different routes of administration.

3.2.2. Reproductive toxicity

Charge Quesiton 2b. The draft assessment concludes that male and female reproductive effects are a human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

The SAB agrees that the data support the conclusion that BaP is a male and female reproductive toxicant through oral and inhalation routes of exposure. A sufficient number of appropriately conducted animal studies are included that demonstrate a functional effect on reproductive endpoints indicative of BaP-related reproductive toxicity and evidence for potential modes of action. The rodent data demonstrate convincingly that BaP affects fertility and fecundity.

Male Reproductive Hazards

The functional effects in male rodents include adverse changes in testes and sperm and hormonal changes. Changes in apical reproductive endpoints (e.g., sperm motility (Mohamed et al. 2010; Chen et al. 2011; Chung et al. 2011; Archibong et al. 2008; Ramesh et al. 2008)) are relevant and useful biomarkers that can be translated for assessing the association of BaP exposure and the potential for adverse effects in humans. Similar changes in sperm quality and fertility have been detected in humans exposed to PAH mixtures (Soares and Melo 2008: Hsu et al. 2006). The exposure to PAH mixtures prevents establishing a causal link between BaP exposure and reproductive toxicity in humans, but the findings are sufficiently consistent with the effects of BaP in rodents to deduce that BaP is a reproductive toxicant in humans.

The SAB recommends that the EPA consider the timing between the treatment with BaP and the measurement of endpoints. Because it is a proliferative tissue, the testis has the potential to recover from exposure to an insult after it is ended. Recovery can include but is not limited to restoration of normal weight based on restoration of spermatogenesis and production of sperm with normal morphology with subsequent waves of spermatogenesis. For sub-chronic studies, it could be informative

to determine if the testes had time to recover in the absence of continued exposure. There is the possibility of an immediate effect from BaP or a PAH mixture that is eliminated with recovery time and which could be dose dependent. The risk is different if recovery is feasible.

The SAB recommends that the EPA consider other hazard endpoints in addition to the classical reproductive hazard endpoints included in the assessment. For example, BaP is mutagenic and mutagenesis in the germline can be detrimental to reproductive health. Therefore, the SAB recommends that the EPA give greater consideration to genotoxic effects on male germ cells as a possible mode of action. The SAB recommends that EPA consider inclusion of additional studies demonstrating that exposure at different life stages (e.g., pre-adult vs adult), can have differential effects on reproductive health. References such as Liang et al. (2012) and Xu et al. (2014) could be used for this purpose.

Female Reproductive Hazards

As noted by the EPA, studies in female rodents that may explain the functional effects of benzo[a]pyrene are limited and contradictory. Benzo[a]pyrene has a direct effect on adult rodent ovarian follicles (Borman et al., 2000; Mattison et al., 1980; Mattison, 1980; Swartz and Mattison, 1985), as well as data presented in Xu et al. (2010). Moreover, a recent study by Einaudi et al (2014) showed that *in vivo* exposure to benzo(a)pyrene induces significant DNA damage in mouse oocytes and cumulus cells. Collectively these aforementioned studies provide insight on the mode of action for benzo[a]pyrene-related decreases in fertility and fecundity. The Xu et al. (2010) study was a low-powered (n=6) mixture study, rather than a typical toxicity study designed to characterize dose-response relationship and target organ toxicity. Other weaknesses are found in this publication including the use of pentobarbital, which is known to affect hormone secretion, and a small number of experimental animals to assess low weight tissues to hormone levels. Guidelines for toxicity studies, including those conducted by the National Toxicology Program, require approximately 10 rats for each gender. The sub-chronic studies by Knuckles (2001; 20 rats/group) and Kroese et al.(2001; 10 rats/group), did not detect changes in ovarian weight revealing the inconsistent outcomes observed in different studies.

In utero exposure of developing females to benzo[a]pyrene provides compelling evidence that there is a sensitive window for exposure to benzo[a]pyrene for the developing ovary (Mackenzie and Angevine, 1981). Benzo[a]pyrene ≥ 10mg/kg affects the developing fetal ovary, resulting in subsequent adult infertility (and in the absence of additional BaP exposure). Because fetal oocyte numbers are fixed prior to birth, as compared with the continual replenishment of sperm after puberty in males, BaP-related loss in oocytes indicates a permanent adverse effect. In humans, tobacco smoke during *in utero* development produces similar effects as benzo[a]pyrene, including effects on subsequent adult fertility. Additional studies cited by the EPA demonstrate that the human ovary is a target for BaP. The results reported from Wu (2010) could be considered relevant to developmental toxicity as well as reproductive toxicity due to early embyonic death, an endpoint also observed in rodent experiments.

General Comments

Germ cells are unique in that they will direct the development of the next generation. The success of the developmental process in producing normal offspring is dependent on the quality of the germ cells and the integrity of their DNA. The genotoxic effects of BaP have not been discussed in the assessment with regard to reproductive toxicity. These genotoxic effects have the potential to result in miscarriages, birth defects and genetic disease – all reproductive hazards. There are no direct studies of the effects of BaP on spermatogonial stem cell mutagenesis, but there is a reference that implicates stem cell mutagenesis

(Olsen et al., 2010). Some papers discuss the mutagenic potential of BaP in somatic cells, but the mechanism is likely the same in germ cells (Young et al., 2014). There are additional references on the effects of BaP on adduct formation, mutagenesis, and gene expression in spermatogenic cells (Verhofstad et al., 2010a; Verhofstad et al., 2010c; Verhofstad et al., 2011). Other papers discuss the processing of BaP adducts during DNA replication and how different polymerases process the damage differently (Starostenko et al. 2014); such differences could contribute to the genotoxic effects in reproductive cells and during development. The Einaudi et al. (2014) study describes DNA damage in oocytes emanating from benzo[a]pyrene exposure. The implication of increased DNA damage and mutagenesis in germ cells causes an increased risk of embryo-fetal death, birth defects and genetic disease among offspring.

Recommendations:

- The SAB recommends that genotoxic and mutagenic aspects of reproductive hazard be addressed, especially as they provide perspective on likely mode of action, or a clear explanation be provided as to why they are not addressed.

- The SAB recommends that the EPA consider additional endpoints (i.e., ovarian and testicular effects) be considered for point of departure/BMD analyses and RfD derivation.

- The SAB recommends that the EPA provide additional clarity as to why certain studies, or parts of studies, are brought forward while others are not.

- EPA should provide context as to the likely applicability of the inflammatory cervical response described in the Gao et al. (2011) study for BMD/RfD generation. EPA may also want to consider if this finding should be categorized under "reproductive effect" or "other toxicity".

- The following reference could be added to sperm effects: Jeng et al., 2015.

- The following references could be added to ovarian effects: Kummer et al., 2013; Mattison 1980; Mattison et al., 1980; Sadeu and Foster, 2011;

- The following reference could be added to mode of action-female reproductive effects: Sadeu and Foster, 2013; Young et al., 2014.

3.2.3. Immunotoxicity

Charge Question 2c. The draft assessment concludes that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

The SAB concludes that the available immunotoxicity data based on exposure of pure BaP in animal models and PAH mixture exposures to humans (coke oven workers) support the conclusion that BaP is a human hazard for the immune system.

The evidence for immunotoxicity in humans is based upon complex PAH mixture exposures. There is no doubt that BaP as a pure chemical can cause suppression of human peripheral blood mononuclear cell (HPBMC) responses at low concentrations *in vitro* (10-100 nM, Davila et al. 1996). However, it is unclear whether the levels of exposure demonstrated to have effects *in vitro* can be achieved from *in*

vivo environmental inhalation exposures or ingestion of cooked foods. Immunotoxicity is caused by a combination of genotoxic (DNA adducts and p53–induced cell death) and non-genotoxic mechanisms (signaling due to AhR activation and oxidative stress, Burchiel and Luster 2001). Some of these mechanisms are similar to cancer initiation and promotion, and there may, in fact, be a relationship between the carcinogenicity of certain PAHs, such as BaP, and their immunotoxicity.

The effects of BaP can vary by dose and time and sometimes lead to complicated non-linear dose-responses resulting in either increased or decreased immune parameters (Burchiel and Luster, 2001). BaP and other similar PAHs have specific structure-activity relationships that are associated with AhR activation and increased P450 CYP1A1, CYP1A2, and CYP1B1 activities. BaP metabolites are likely responsible for the immunotoxicity seen *in vivo*. Thus, complicated dose-response relationships can be seen, that result from the actions of different metabolites of BaP (e.g., BP-diol-epoxides, vs BP-quinones).

Human Studies

EPA has captured the key evidence that makes a strong case for the immunotoxicity of BaP in humans, which are all based on exposure to PAH mixtures.

Szczeklik et al. (1994) reported decreased serum immunoglobulins (Igs) in coke workers with inhalation exposures. Zhang et al., (2012) studied 129 coke oven workers (compared to 37 warehouse controls) for early and late apoptosis (Annexin V/PI) in HPBMC. The concentrations of BaP were 10-1,600 ng/m^3 in the working environment; 2.78-3.66 ng 1-hydroxypyrene (1-OHP) were measured in urine. Karakaya et al. (1999) found an increase in serum Ig, which is not consistent with Szczeklik et al. (1994), and may be associated with a difference in exposure dose and/or duration.

Winker et al. (1997) is an immune function and phenotype study of HPBMC comparing old and new coke facilities. This study shows a depression of T cell activation, and the results are very compelling. Karakaya et al. (2004) also showed decreased T cell proliferative responses in asphalt and coke workers.

Because BaP is present in cigarette smoke, cigarette smoke studies are relevant for consideration. Numerous cigarette smoking studies have demonstrated immune suppression, but the interpretation of these effects is complicated by the strong action of nicotine, which in itself is immunosuppressive. Therefore the inclusion of cigarette smoking studies is not recommended for this IRIS assessment. Cigarette smoking can also be an important confounder for other environmental cohort studies, and must be examined as an independent variable (Karayaka et al. 2004).

Animal Studies

EPA focuses on De Jong et al. (1999) and Kroese et al. (2001) studies in rats with the toxic endpoint being thymic atrophy at 90 mg/kg to derive its RfD. However, these studies did not employ immune function studies that are known to be more sensitive. EPA acknowledged that thymic atrophy may not be a reliable indicator of immunotoxicity (page 2-5, line 19).

Most immunotoxicity animal studies utilize mouse models (not rat) and they rely upon sensitive functional assays, such as the T-dependent antibody response (TDAR). In this assessment, the EPA has acknowledged the mouse immune function studies (page 1-38, lines 20-28), but they have not been included in the RfD calculation, presumably because these studies employed parenteral routes of

administration and did not utilize adequate numbers of animals per group and a sufficient number of doses for evaluation. This is a common limitation of studies designed for assessing mechanism of action rather than regulatory needs.

The dose required to produce thymic atrophy is known to be quite high in mice and rats compared to that required to alter immune function (Luster et al. 1992). There is an overall consistency of findings for BaP immunotoxicity in mice and some rat strains. Temple et al. (1993) showed decreased IgM response and plaque forming cell (PFC) in mouse spleen at 5, 20, 40 mg/kg and F344 rats at 10 and 40 mg/kg 14 days administered via subcutaneous injection, but the use of the rat model is limited by the lack of a substantial immunotoxicity database.

Important structure activity relationships established early on by Dean et al (1983) showed suppression of phytohemagglutinin (PHA)-induced T cell proliferation response of mouse spleen cells following exposure of mice to 50 mg/kg BaP, but not by benzo[e]pyrene (BeP), a non-carcinogenic congener. In mice, Ladics et al. (1992) showed that BaP metabolites are responsible for suppression of the TDAR in mouse spleen.

Immune function tests indicate that BaP is suppressive and should result in increased risk of infections and perhaps cancer. This is evidenced by Munson et al. (1985) who showed a decreased resistance to Strep, Herpes, and B16 melanoma by BaP but not by BeP. Influenza infectivity was not affected by BaP and Listeria resistance was increased, thus demonstrating the complicated dose responses discussed above. Kong et al. (1994) also demonstrated decreased lung resistance to tumor cell challenge in Fischer 344 (F-344) rats following intratracheal administration of BaP.

Collectively, these animal studies provide strong evidence that BaP suppresses immune function leading to adverse consequences for host resistance to infections. The limitation of most of these studies with respect to assisting EPA in establishing an RfD based on immune function tests is that adequate exposure dose ranges were not used and parenteral exposure routes and short study durations are less pertinent for RfD derivation as described in section 3.3. of the preamble to the assessment.

Developmental Immunotoxicity

Developmental immunotoxicity is not well-addressed in the assessment. The assessment derived an RfD based upon developmental exposures. Although BaP was found to produce alterations in T cell development by several investigators (Urso and Gengozian 1982, 1984; Urso and Johnson 1987; Rodriguez et al. 1999), these studies were limited by the use of a single high dose (150 mg/kg) of BaP. Holladay and Smith (1994) found that 50 mg/kg total cumulative doses were able to decrease thymus cellularity and inhibit T cell development in the thymus of mice exposed gestationally. A decreased number of spleen cells was also seen by these investigators (Holladay and Smith, 1995).

In addition to the evidence that BaP alters T cell development *in utero* and in adults, there is also evidence that BaP alters B cell development in the bone marrow of adults (Hardin et al., 1992). These effects may be dependent on the expression and activity of the AhR.

It is likely that the developing immune system may be one to two orders of magnitude more sensitive to BaP exposures than adult exposures (Dietert et al., 2000, 2006; Leubke et al., 2006; WHO, 2012). It is

unclear whether the application of uncertainty factors can address these concerns regarding the inadequacy of the database. It is generally well known that developmental immunotoxicity is produced at much lower doses than those required to produce immunotoxicity in adults. However, this may not be well documented for BaP in the present literature citations used for assessment.

Recommendations

The BaP assessment could be improved by a well-defined, unified approach for immunotoxicity risk assessment (e.g., through a guidance document), that identifies sensitive biomarkers of exposure and effect for the immune system of animals and humans.

- EPA should look for evidence of increased infections in cohorts as a demonstrated health effect of BaP exposure, which would be indirect evidence of immunotoxicity.

- EPA should consider developing Guidelines for immunotoxicity assessment to standardize risk assessment and to identify data gaps, as has been done by WHO (2012).

- *In vitro* human PBMC studies should be included that support an understanding of mechanisms of action; EPA should utilize mechanism of action data more fully in their risk assessment.

- BaP exposures are relatively high in woodsmoke inhalation studies, but there are few immunotoxicity studies (Burchiel et al. 2005); immunotoxicity resulting from woodsmoke inhalation and other sources of human environmental exposure to BaP should be considered by EPA.

3.2.4. Cancer

Charge Question 2d. The draft assessment concludes that benzo[a]pyrene is "carcinogenic to humans" by all routes of exposure. Do the available human, animal, and mechanistic studies support this conclusion?

The SAB finds that the EPA has demonstrated that BaP is a human carcinogen in accordance with the *Guidelines for Carcinogen Risk Assessment* (USEPA, 2005a). This conclusion was based primarily on animal studies and mechanistic data, with strong support from an excess of lung cancer in humans who are exposed to PAHs, but not to BaP alone. This conclusion is consistent with the evaluations by other agencies, including the World Health Organization International Agency for Research on Cancer (2010) and Health Canada (2015). Detailed consideration of the EPA criteria for whether or not a compound is considered a human carcinogen, as applied to BaP, follows.

EPA Criterion 1 - The compound in question is "Carcinogenic to Humans" when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.

The SAB agrees that occupational studies strongly indicate that PAH mixtures are carcinogenic to humans. Relevant occupations include, but are not limited to, chimney sweeps and workers in coke oven, iron, steel, and aluminum production. Other sources of significant human PAH exposure associated with cancer include chronic ingestion of PAH-contaminated food, and chronic inhalation of fumes from both cooking food and indoor heating with particular kinds of coal. However, as the EPA BaP assessment states, in the arena of human exposure, it is not possible to separate BaP from other carcinogenic PAHs. Therefore, from the epidemiologic studies there is no direct evidence that BaP alone

is carcinogenic. Because there is the assumption that BaP is always a component of the PAH mixtures that humans are exposed to, a logical conclusion is that BaP alone is likely to be a human carcinogen based on the epidemiologic evidence. However, this assumption alone is likely not sufficient to satisfy the first EPA criterion.

The BaP assessment focused on lung, bladder and skin cancers, but these are not the only organs for which PAHs are carcinogenic. There is strong evidence for an association between PAH-exposure in heavily char-broiled meat (Rothman et al., 1993) and colon adenoma risk (Sinha et al., 2005). In addition there are strong associations between PAH-DNA adduct formation, cooked meat ingestion and colon adenoma risk in the same population (Gunter et al. 2007).

The SAB suggests that the EPA reconsider the requirement for individual monitoring data (Tier 1 studies) in choosing to present epidemiological studies, because some important papers have been overlooked (see Appendix B). The Supplemental Information document summarizes six human studies (Table D-33), which evaluated BaP-induced DNA adducts in humans. This is a small fraction of the available studies that employ chemical class-specific methods to measure PAH-DNA and BPdG adduct formation in human tissues. It is possible that some epidemiological studies have been omitted by the EPA for lack of individual personal monitoring data. One could argue that for biomarker association studies, and for establishing or supporting hazard identification in a workplace known to be polluted, personal monitoring is not necessary. The presence of high ambient levels of BaP and/or PAHs, high levels of urinary 8-hydroxy-pyrene, and/or high levels of BPdG are all strong indicators of exposure. However, personal monitoring would be necessary for using epidemiological data to support dose-response calculations.

There are a series of human epidemiological studies, involving cohorts of individuals, where subjects have been stratified into quartiles or quintiles for their PAH-DNA adduct level (using chemical class-specific methods). These studies have reported significant increases in cancer risk in individuals having the highest PAH-DNA adduct levels, compared to those having the lowest levels. Compiling this data into a table in the Supplemental information would be very useful (see: Kyrtopoulos, 2006; and Poirier, 2012).

The issue of the lack of an excess of skin tumors observed in most studies of therapeutic use of coal tar was discussed (Jones et al. 1985; Muller and Kierland 1964). The SAB agrees with the EPA that many of these studies suffer from small sample size, inadequate followup and a large potential for exposure misclassification. In addition, the skin of psoriasis patients who receive these treatments is not normal skin, which may have affected the outcome of the studies. The limitations of these studies make them largely uninformative with regard to the question of whether BaP induces skin cancer in humans. The historic studies of an excess of scrotal cancers in chimney sweeps, and more recent studies demonstrating an excess risk in asphalt workers, are consistent with exposure to BaP being a risk factor for skin cancer.

EPA Criterion 2 - The compound in question can be considered "Carcinogenic to Humans" when there is a lesser weight of epidemiological evidence but when all of the following conditions are met:

a) *strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association*

b) *extensive evidence of carcinogenicity in animals*

c) *the mode(s) of carcinogenic action and associated key precursor events have been identified in animals*

d) *there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information*

The SAB agrees that the sum total of the mechanistic data show that all four of the required conditions are met. Therefore, based on epidemiologic studies of cancer in humans and animal models, and on mechanisms of action determined in both species, strong evidence of key precursor events related to BaP exposure and found in humans indicates that BaP can be considered a human carcinogen.

The SAB agrees that BaP is metabolized/activated through three separate pathways: the diol-epoxide pathway, the radical cation pathway and the *o*-quinone pathway. Furthermore, the SAB agrees that BaP-induced tumors arise primarily through a mutagenic mode of action resulting from BaP-induced DNA damage. Several studies over the last decade have shown that challenge of primary and transformed cells with BaP increases retrotransposition of Long Interspersed Nuclear Element-1 (L1) (Stribinskis and Ramos 2006). Long interspersed nuclear element–1 (L1) retrotransposons are highly active mobile repetitive elements abundant in the human genome (Ramos et al. 2013). Retrotransposition of L1 induces DNA strand breaks, increased frequency of recombination and insertion mutations directly linked to various types of cancers (reviewed in Beck et al. 2011), as well as disruption of local genome architecture and loss of transcriptional control of neighboring genes (Raiz et al. 2012). As such, in addition to the mutational activity of reactive electrophilic metabolites of BaP, the carcinogenic activity of BaP may involve genetic and epigenetic events mediated by L1 reactivation (Teneng et al. 2011).

The most chemically-stable DNA adducts of BaP are formed via the diol-epoxide pathway and persist in human tissues for many years (VanGijssel et al. 2004.) Much of the DNA damage generated by the radical cation and *o*-quinone-ROS pathways is unstable, and some additional stable DNA damage (8-OH-dG, ROS) is also caused by xenobiotics other than benzo[*a*]pyrene. The steps connecting benzo[a]pyrene exposure and tumor formation by a mutagenic mechanism have been studied most completely in the diol-epoxide pathway. However, because BaP is a complete carcinogen, the SAB emphasizes that the mechanism of action must include both the initiating (mutagenic) effects and the promoting effects. The promoting effects appear to occur largely through the radical cation and quinone metabolic pathways, which increase cell proliferation, generate ROS and activate various growth factors and signaling pathways (Burdick et al. 2003).

The SAB suggests that EPA could strengthen the statements in the assessment that describe the pathway linking benzo[a]pyrene exposure to tumor formation. The SAB recognizes that there is an overwhelming literature available, and sorting out the critical original papers is daunting. The following is a series of findings that highlight the critical steps in the diol-epoxide pathway connecting exposure to tumorigenesis via a mutagenic mode of action. Statements are supported by original literature. This information might clarify/enhance the statements in Table 1-17 on page 1-75, "Experimental support for the postulated key events for mutagenic mode of action".

- Benzo[a]pyrene is metabolized/activated via the 7,8-diol to the diol-epoxide (r7,t8-dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene or BPDE)
    - Sims, P. et al, Metabolic activation of benzo[a]pyrene proceeds by a diol-epoxide, Nature 252:236-327, 1974.

- King, H. W. S. et al., 7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene is an intermediate in the metabolism and binding to DNA of benzo[a]pyrene, Proc Natl Acad Sci USA 73:2679-2681, 1976.

- BPDE interacts with the N2 position of guanine to form the stable r7,t8 ,t9-trihydroxy-c-10-(N^2-deoxyguanosyl)-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPdG) adduct.
  - Daudel, P., Fluorescence spectral evidence that benzo[a]pyrene-DNA products in mouse skin arise from diol-epoxides, FEBS Letters, 57:250-253, 1975.
  - Jeffrey, A.M. et al., Benzo[a]pyrene-nucleic acid derivative found in vivo: structure of a benzo[a]pyrene-tetrahydrodiol epoxide-guanine adduct. Journal of the American Chem. Soc. 98:5714-5, 1976.

- BPdG forms in human cells and in mouse skin.
  - Grover, P.L.et al., The involvement of a diol-epoxide in the metabolic activation of benzo[a]pyrene in human bronchial mucosa and in mouse skin, Int. J. Cancer 18:1-6, 1976.
  - Osborne, M.R. et al., The reaction of 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene-9,10-oxide with DNA in relation to the benzo[a]pyrene-DNA products isolated from cells. Chem.-Biol. Interactions 13:343-348, 1976.

- The BPdG adduct is mutagenic. Site-specific studies linked mutation hotspots with regions of inefficient BPdG repair in modified DNA.
  - Wei, D. et al., Site-specific rates of excision repair of benzo[a]pyrene diol epoxide adducts in the hypoxanthine phosphoribosyltransferase gene in human fibroblasts: Correlation with mutation spectra. Proc. Natl. Acad. Sci. USA 92:2204-2208, 1995.

- Formation of the BPdG adduct in an oncogene can mutate and activate that oncogene. Mutated clones of the c-Ha-*ras* oncogene were formed as a result of *in vitro* reaction of the BPDE with the c-Ha-*ras* proto-oncogene. The resulting activated c-Ha-*ras* oncogene caused malignant transformation in NIH 3TC cells.
  - Marshall, C.J. et al., Activation of c-Ha-*ras*-1 proto-oncogene by *in vitro* modification with a chemical carcinogen, benzo[a]pyrene diol-epoxide. Nature 310:586-589, 1984.

- BaP caused dose-related increases in forestomach tumorigenesis and forestomach BPdG levels during chronic lifetime (2 yr) feeding in mice.
  - Culp, S.J. and Beland, F.A. Comparison of DNA adduct formation in mice fed coal tar or benzo[a]pyrene. Carcinogenesis 15:247, 1994.
  - Culp, S.J. et al., A comparison of the tumors induced by coal tar and benzo[a]pyrene in a 2 year bioassay. Carcinogenesis 19:117, 1998.

- Reduction in levels of the benzo[a]pyrene-7,8-diol metabolite, BPdG formation and tumor formation was observed in mice treated with benzo[a]pyrene in the presence of the chemopreventive agent benzyl-isothiocyanate.
    - Sticha, K.R.K. et al., Effects of benzyl isothiocyanate and phenyl isothiocyanate on benzo[a]pyrene metabolism and DNA adduct formation in the A/J mouse. Carcinogenesis 21:1711, 2000.

- First detection of a chemically-characterized BPdG adduct in human tissue DNA.
    - Manchester, DK; Weston, A; Choi, J-S; Trivers, GE; Fennessey, PV; Quintana, E; Farmer, PB; Mann, DL; and Harris, CC. (1988) Detection of benzo[a]pyrene diol-epoxide-DNA adducts in human placenta. *Proc. Natl. Acad. Sci. USA.*, 85: 9243-9247.

- In 39% of 705 human tissue DNA samples it was possible to detect the presence of BPdG adducts, determined by chemical-specific methods (Boysen and Hecht). In addition, PAH-DNA adducts were localized in multiple human tissues by immunohistochemistry (Pratt et al.)
    - Boysen, G. and Hecht, S.S. Analysis of DNA and protein adducts of benzo[a]pyrene in human tissues using structure-specific methods. Mutation Research 543:17-30, 2003.
    - Pratt, M.M. et al., Int. Journal of Environmental Research and Public Health 8:2675-2691, 2011.

- PAH exposures in humans are associated with a high frequency of GC→TA transversion mutations, however this type of mutation can be caused by other xenobiotic agents and therefore occurrence does not always provide a direct link to benzo[a]pyrene exposure.
    - Hussain, S.P. et al., Mutability of p53 hotspot codons to BaP diol epoxide (BPDE) and the frequency of p53 mutations in nontumorous human lung. Cancer Research 61:6350-6355, 2001.

Critical to our understanding of the published values for human BaP-induced DNA adducts and PAH-DNA adducts is knowledge of what is being measured by a specific assay. The gold standard is the use of structure-specific methods (Boysen and Hecht, 2003.) Other assays have compound-class specificity. For example, the various antibody-based methods (ELISA and immuno-histochemistry) employ monoclonal or polyclonal antibodies (termed BPDE-DNA antisera) raised against BaP-modified DNA. These antisera cross-react with a family of carcinogenic PAHs bound to DNA. When evaluating human tissue DNA, the data are expressed as "PAH-DNA adducts" because of the cross reactivity to DNA samples modified with multiple carcinogenic hydrocarbons. Other assays are not benzo[a]pyrene or PAH specific. For example with ^{32}P-postlabelling, which detects adducts of many different chemical classes, it is not possible to identify BPdG in human samples. Choice of an assay will impact the validity, reliability and conclusions obtained from a particular study. In the original literature there is often confusion in the use of nomenclature. The Toxicological Review and Supplemental information would be more user friendly with the addition of a table describing the characteristics and nomenclature of the various methodologies used for BPdG and PAH-DNA adduct measurements.

The SAB found some of the text on page 1-72 of the assessment to be vague or inaccurate. For example, line 25 "These results are consistent with evidence that BaP diol epoxide is reactive with guanine bases in DNA…." This statement is vague, despite the fact that there is actual experimental evidence in the

literature that would allow a more precise statement. In addition the sentence starting with "Supporting…." on line 33 of that page, the statement that "…benzo[a]pyrene diol epoxide (specifically[+]-anti-BPDE) is more potent than BaP itself…in producing lung tumors in newborn mice following i.p. administration" is not correct (and is not supported by a reference). Despite the fact that it is direct-acting, the diol-epoxide is too labile to be carcinogenic *in vivo*.

3.2.5. Other types of toxicity

Charge Question 2e. The draft assessment concludes that the evidence does not support other types of noncancer toxicity as a potential human hazard. Are there other types of noncancer toxicity that can be credibly associated with benzo[a]pyrene (BaP) exposure?

The potential hazards identified and discussed in Section 1.1.4 are forestomach toxicity, hematological toxicity, liver toxicity, kidney toxicity, cardiovascular toxicity, and (adult) nervous system effects. Overall, EPA concluded that the available evidence does not support these noncancer effects as potential human hazards (Section 1.2.1). The SAB recommends that the basis for arriving at this conclusion be expanded for each of these health endpoints. The current text does not provide an adequate rationale for why the evidence does not support the listed effects as potential human hazards. EPA needs to clarify whether this conclusion is due to insufficient data, inconsistent data, or sufficient data to conclude that these health endpoints are not sensitive endpoints.

EPA has organized the summaries of human and animal studies in tables by target organ or system effect (e.g., kidney toxicity; nervous system effects), and animal study tables include helpful information on study design (species, strain, sex, number per group, dose levels, route of administration and dosing regimen/duration) and study results. Additional context regarding the overall study results is often needed to interpret the findings for a specific endpoint, including available toxicokinetic information for the relevant dose range, if organ weight changes were or were not accompanied by histopathological changes; and observations that inform the general health status of animals under study.

With respect to the health endpoints discussed in section 1.1.4, the SAB concludes that the evidence presented does not support liver, kidney, and hematological effects as human hazards; the EPA's rationale for those conclusions is incompletely described and the conclusions depend on the literature search and study selection process, which was not considered to be sufficiently comprehensive to identify all potential hazards credibly associated with BaP exposure (see response to Charge Question 1 – Literature Search/Study Selection and Evaluation). Notably, the list of search terms used indicates that no queries were made that included the term "cardio" (i.e., cardiotoxicity; cardiovascular; cardiopulmonary), "vascular," "athero*," etc. Similarly in the literature search secondary refinement, it is noted that certain potential target organs (e.g., heart, liver, and kidney) were not included in the search terms. Thus it is unclear that the assessment of all potential targets identified in the hazard identification section (specifically section 1.1.4) was comprehensive. Moreover, it is unclear how the information obtained from mechanistic studies was integrated into the assessment of hazards.

The SAB's conclusion regarding target organ toxicities reviewed by EPA is summarized below:

Forestomach: The available evidence presented does not support EPA's conclusion that forestomach toxicity in rodents is not a potential human health hazard.

The document should be internally consistent regarding the human health hazard of forestomach toxicity. The EPA did not consider human relevance to be an appropriate basis for excluding the credible evidence of forestomach toxicity associated with BaP exposure, noting that humans do not have a forestomach but do have similar squamous epithelial tissue in their oral cavity. This conclusion is at odds with the overall conclusion for this section that the available evidence does not support forestomach effects as representing a potential human hazard.

The decision to not consider forestomach toxicity further for dose-response analysis and the derivation of reference values, as explained in section 1.2.1 "Weight of Evidence for Effects Other than Cancer," should not be used as a justification for excluding forestomach toxicity as a hazard credibly associated with BaP exposure. Forestomach toxicity may reflect a tumor-promoting key event in the tumorigenic mode of action, and thus reflect part of a combination mode of action discussed by the EPA in the section "other modes of action."

For these reasons, forestomach toxicity is credibly associated with BaP exposure, so it is reasonable to identify it as such in the hazard identification section of the document. The SAB recommends that EPA consider factors identified in IARC (2003) such as mode(s) of action and influencers of target tissue residence time (viz., method and vehicle of BaP administration) in addressing the predictive value for humans of forestomach effects in rodents.

Hematological toxicity: The available studies presented support the conclusion that hematological toxicity is not a potential human hazard.

The summary of hematological toxicity is well done. The evidence provided for hematological toxicity appears to be limited and suggests only a marginal effect on hematological parameters as the magnitude of the alterations may not be biologically significant. The data presented suggest that dose rate may influence blood cell parameters, but not in a reproducible fashion. Changes are minimal or statistically insignificant at all but the highest dose levels (repeated oral dosing of 90 or 100 mg/kg-day). Based on the evidence presented, the SAB agrees with the conclusion that the studies presented do not provide convincing evidence that hematological effects are a human hazard of BaP exposure.

Liver toxicity: The available studies presented support the conclusion that liver toxicity is not a potential human hazard.

The evidence provided for liver toxicity appears to be limited and suggests that while effects may be observed at higher exposure levels it does not appear to be a sensitive health endpoint. The studies described in this section reporting noncancer effects of BaP to the liver can be summarized as identifying reproducible organ weight changes (all three studies) without associated histopathology in two studies. In the third study, increased liver oval cell hyperplasia was reported only at the highest dose level (90 mg/kg-day) following 35-day gavage dosing (DeJong et al. 1999). EPA should clarify whether histopathology evaluations of the liver were performed by Knuckles et al. (2001). Based on the evidence presented, the SAB agrees with the conclusion that these studies do not provide convincing evidence that noncancer liver effects are a human hazard resulting from BaP exposure. The results of Wester et al. (2012) (not cited in the assessment) should also be addressed which may provide additional support for this conclusion.

Kidney toxicity: The studies presented support the conclusion that kidney toxicity is not a potential human hazard; however, adult and developmental renal toxicity are not fully addressed in the assessment.

In the three studies discussed by EPA, there is no consistent finding indicative of kidney toxicity. The evidence provided for kidney toxicity therefore appears to be limited and suggests that while effects may be observed at higher exposure levels, it does not appear to be a sensitive health endpoint. However, the SAB has identified relevant references regarding the effects of BaP on renal function in rats (Alejandro et al. 2000; Parrish et al. 2002; Nanez et al. 2005; Valentovic et al. 2006), and the intrauterine effects of BaP on kidney morphogenesis and late onset renal disease (Nanez et al. 2011). The SAB recommends that these studies be reviewed to determine whether there is convincing evidence that noncancer kidney effects are a developmental and/or adult human hazard resulting from BaP exposure.

Cardiovascular toxicity*:* The available studies do not support EPA's conclusion that cardiovascular toxicity is not a potential human hazard and further explanation is needed as to the rationale reaching this conclusion.

The evidence provided for cardiovascular toxicity suggests potential toxicity at low dose levels, recognizing that the data are too limited to be utilized quantitatively. It is not clear why evidence pertaining to cardiovascular toxicity is not included in Table 1-9, and whether the designs of the animal studies reviewed were suitable to identify adverse cardiovascular effects. There are multiple modes of action by which chemicals may adversely impact the cardiovascular system, and it is unclear if different lines of evidence (i.e., mechanistic, animal and human) were integrated for hazard identification. Since cardiovascular effects were identified in rats and mice following gestational exposures to BaP, EPA should address whether such findings should be considered as part of the weight of evidence for the cardiovascular system as a potential adult target of BaP exposure. Although limited, the two epidemiology studies cited (Burstyn et al. 2005; Friesen et al. 2010) lend credence to possible human relevance of this endpoint.

The SAB concludes that the literature search was not sufficiently comprehensive to identify studies relevant to addressing the identification of cardiovascular system toxicity of BaP exposure (see comments to charge question 1 – literature search/study selection and evaluation). Several studies showing an influence of BaP on the severity and progression of atherosclerotic plaques in animal models (as cited by Oesterling et al. 2008 – not included in this section) are not addressed. Other studies to be considered as part of the weight of evidence evaluation, but not cited in this section are Knappen et al. (2007) and Yang et al. (2009) which address the induction of atherosclerosis by BaP in rodents; and Aboutabl et al. (2009, 2011), which examine cardiac hypertrophy and cardiac biomarkers after BaP exposure. The induction of inflammatory cytokines by BaP (e.g., N'Diaye et al. 2009 – not cited; and N'Diaye et al. 2006 – cited on p 1-77) should be included as part of the weight-of-evidence discussion of cardiotoxicity. Other relevant recently published articles include Gan et al. (2012), Uno et al. (2014) and Javasundara et al. (2015).

Gan et al. (2012). Biomed Environ Sci 25(5):549-56. Effects of BaP on the contractile function of the thoracic aorta of Sprague-Dawley rats.

Jayasundara et al. (2015). Tox Sci 143(2):469-81. AHR2-Mediated Transcriptomic Responses Underlying the Synergistic Cardiac Developmental Toxicity of PAHs.
Uno et al. (2014). Toxicology 316:34-42. Protective role of cytochrome P450 1A1 (CYP1A1) against benzo[a]pyrene-induced toxicity in mouse aorta.

The SAB recommends EPA address the references that are missing. If they were excluded, the basis for their exclusion should be provided. If not intentionally excluded, the missing references should be included as part of the weight of evidence evaluation. EPA should be explicit regarding the rationale for concluding that the available evidence either does or does not support cardiovascular system toxicity as a potential human hazard.

Adult nervous system toxicity: The available studies do not support EPA's conclusion that adult nervous system toxicity is not a potential human hazard.

Further explanation is needed as to the rationale for concluding that the available evidence does not support adult nervous system effects as a potential human hazard. The SAB notes that although EPA's draft assessment concludes in Section 1.2.1 that adult nervous system is not a potential human target, this conclusion was not explicitly stated in Section 1.1.4, where EPA indicates that the evidence for "forestomach, liver, kidney, and cardiovascular system, as well as alter hematological parameters" (page 1-44) does not support potential human hazards for these endpoints. "Nervous System Effects," however, are discussed in Section 1.1.4, which ends with the statement "These data suggest that benzo[a]pyrene exposure could be neurotoxic in adults; however, only limited data are available to inform the neurotoxic potential of repeated subchronic or chronic exposure to benzo[a]pyrene via the oral route (Table 1-9)" (p.1-49). This section should be expanded to include a more rigorous evaluation of the adult neurotoxicity evidence, especially since EPA concludes that developmental neurotoxicity is a potential human hazard. EPA should clarify the conclusion with respect to adult neurotoxicity and be consistent in Sections 1.1.4 and 1.2.1 of the assessment.

The evidence provided for adult neurotoxicity suggests potential toxicity at low dose levels, recognizing that the data are too limited to utilize quantitatively for oral exposures. Decrements in short term memory were reported in two studies of workers exposed occupationally to PAH mixtures containing BaP (Niu et al. 2010; Qiu et al. 2013), lending possible credence to the human relevance of this endpoint.

The SAB notes that Table 1-9 includes only two studies informing the neurotoxic potential of BaP exposure in adult animals following subchronic or chronic oral exposures. If this is the case, EPA should indicate in the title of the table that only oral studies are included, because many more studies are discussed in the text. Since hazard identification does not rely only on repeated subchronic or chronic exposure scenarios alone, EPA might consider developing a separate summary table just for neurotoxicity studies that includes Grova et al. 2007, 2008; Saunders et al. 2001, 2002, 2006; Liu et al. 2002; Maciel et al. 2014; Chen 2011; Qiu et al. 2011; Xia et al. 2011; and Bouayed et al. 2012. This summary table should include information on route, dose levels, and dose-response relationship, including both positive and negative findings. Considering the relatively low doses in laboratory animals at which behavioral alterations were reported, the rationale for not considering the adult nervous system as a potential human target is unclear.

The section on adult neurotoxicity was not sufficiently rigorous in the analysis of oral neurotoxicity studies in either the text or in the table. Bouayed et al. (2012), an oral study, was not included on Table 1-9. EPA may have mistaken this as an i.p. exposure study. EPA should report the negative finding on motor activity, and indicate that there were mixed results, rather than a decreased depressive-like activity. EPA should clarify that there was no dose-response relationship (effects at 0.02 and 0.2, but not at 2 or 20 mg/kg/day), and that these effects could be acute effects, because the behavioral tests were conducted 60 minutes after gavage dosing.

EPA indicates that Bouayed et al. (2009) reported an increase in aggressive behavior and consummatory sexual behavior in mice treated with 0.02 mg/kg-day, but should indicate in the text that there were no effects at 0.2 mg/kg-day (the highest dose tested). EPA links this increase in aggressive behavior with decreased "anxiety" on the open-field test (pp. 2-3), yet the dose-response pattern is not consistent. EPA should be more cautious about interpreting these findings, because (a) the significance of four vs. two "attacks" is not clear, (b) Bouayed et al. (2009) provides no clear definition of how "attacks" were defined and distinguished from other social behaviors such as "play," and (c) the observers were not kept unaware of the treatment level.

The Grova et al. (2008) paper is an i.p. study that is not included in Table 1-9, presumably because Table 1-9 includes only oral studies. EPA relates the increased time in the open arm of the plus maze in adult animals (Grova et al. 2008) to that observed in offspring (Chen et al. 2012) (p 2-3). Yet EPA does not indicate (pp. 1-49 and 2-3) that this was a high-dose effect that occurred at 200 mg/kg (i.p.) and not at the lower doses of 0.02–20 mg/kg.

As reviewed in the EPA assessment, nervous system toxicity was assessed in animal studies where BaP was administered starting at weaning, adolescence, or to adult rodents. The SAB concurs with the EPA that these represent additional types of non-cancer BaP toxicity. However, the SAB suggests that the EPA include these in its overall assessment of BaP as both a developmental and adult neurotoxic agent. It was not clear in the assessment what the cutoff was for placing a study in the developmental versus non-developmental category given that there are prenatal, neonatal, weaning, and adolescent exposure studies, all of which are developmental in one sense or another even apart from the adult neurotoxicity exposure studies. The EPA assessment clearly included the prenatal and early postnatal studies in the developmental neurotoxicity section, but placed the weaning (starting exposure at P21) and adolescent (starting exposure at P28) in the "other" non-cancer nervous system section. Further justification of the boundaries would be useful.

The SAB recommends that EPA be explicit as to the rationale for concluding that the available evidence either does or does not support adult nervous system effects as a potential human hazard.

Other Toxicity:

In addition, the SAB identified adult and developmental pulmonary toxicity as noncancer endpoints that can be credibly associated with BaP exposure, but were not identified in the draft assessment.

Adult and developmental pulmonary toxicity are not well addressed in the document. The SAB identified references in regard to the effect of maternal exposure to BaP on fetal development, and recent epidemiological studies that suggest an association between dietary BaP intake and lower birth weight in children (Duarte-Salles et al. 2010, 2012, 2013). Also, there is little emphasis on the effects of

BaP on non-cancer pulmonary toxicity. Thakur et al. (2014) present evidence that maternal exposure of mice to BaP leads to increased susceptibility of newborn mice to hyperoxic lung injury and chronic lung disease (CLD). Supplemental oxygen therapy is frequently encountered in premature infants and very low birth weight infants, and hyperoxia contributes to the development of bronchopulmonary dysplasia (BPD), also known CLD, in these infants. Maternal smoking is one of the risk factors for preterm birth and for the development of BPD. This literature describing the effect of BaP on pulmonary toxicity in infants as well as adults should be included.

3.3. Dose-response analysis

In section 2 of the draft assessment, the EPA uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with benzo[a]pyrene exposure in section 1, then proposes an overall toxicity value for each route of exposure. The SAB comments on the EPA analyses in the sections that follow.

3.3.1. Oral reference dose for effects other than cancer

Charge Question 3a. The draft assessment proposes an overall reference dose of $3x10^{-4}$ mg/kg-d based on developmental toxicity during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?

The SAB finds that developmental endpoints, and in particular, neurodevelopmental endpoints are, in principle, an appropriate basis for deriving an RfD for benzo[a]pyrene. However, the EPA has not made a sufficiently strong case that the available developmental endpoints are the most appropriate non-cancer endpoints for setting an RfD, or that among the available neurodevelopmental endpoints, the observed results from the elevated plus maze test in Chen et al. (2012) are the most appropriate results.

With respect to developmental toxicity as the most appropriate category of non-cancer effects, the SAB suggests that EPA give more consideration to the available reproductive outcomes, including cervical hyperplasia and cervical inflammation in Gao et al. (2011), and at least provide a firmer justification for not selecting these as critical endpoints. In addition the EPA should better explain the reasons for not modeling immunotoxicity (IgM, IgA) endpoints.

With respect to the choice of specific neurodevelopmental endpoints, the SAB notes that there are several important positive aspects to the Chen et al. (2012) study. These include: adequate numbers of litters (40 litters, 10/dose group) were used; there was a well-defined dose-response for several behavioral outcomes; the overall study presented multiple and well characterized tests; and the subjective tests were conducted with observers blind to treatment level. However, the SAB also identified several potentially significant negative aspects the study design and data analysis in Chen et al. (2012) that were either not addressed or were not fully considered in the EPA assessment. These include: potential dam and pup stress from repeated rotation of dams; potential nurturing bias against high dose pups based on smell and/or behavioral differences especially following gavage doses; and the total number of dams used and timing (e.g., litters redistributed to other dams who gave birth within 24 hrs of each other) to achieve 40 litters of 4 M and 4F divided into 10 litters per track was not described.

Presumably, all 40 litters were not born in one day, so the details on how this was achieved, including use of >40 litters initially, so that pups are exactly the same age in each litter are a critical part of study design that can impact study outcome and interpretation of data.

In addition, although the BMD approach employed by the EPA for deriving the POD is not dependent on the specific statistical tests used for group comparisons, the overall weight of evidence and evaluation of this study is based on the original statistical analysis using the Least Significant Difference (LSD) post hoc test. This test appears to be statistically inappropriate in this context.

Given these concerns, the SAB recommends that the EPA should specifically consider the overall picture of neurodevelopmental impact from all of the neurodevelopmental endpoints in Chen et al. (2012), including plus maze, reflex, locomotor activity and water maze to justify and support the choice of the critical endpoint. In particular, the SAB suggests that EPA reconsider or provide stronger justification for not using escape latency from the Morris water maze. This endpoint appears to be the most stable behavioral difference that was repeated 4 days for 2 separate tracks (cohort) of animals. The EPA is correct that this effect is not a learning or memory effect due to difference in baseline from day 1, but it is some indication of an effect (even if that effect is a developmental effect on locomotion). EPA should explain how the BMD was calculated for escape latency since there are 4 different days for each track and each sex.

The SAB agrees with EPA's decision not to further consider the Xu et al. (2010) study, but given its drawbacks, the SAB concludes that this study should not have been included in Table 2-2.

With respect to the application of uncertainty factors (UFs), the assessment stated that the application of a full UF of 10 to the POD from the EPM for the animal to human extrapolation in Chen et al. (2012) was needed because a $bw^{3/4}$ adjustment in deriving the POD for that endpoint. EPA stated that this was because the allometric $bw^{3/4}$ adjustment is not appropriate for extrapolating from neonate animal to adult humans. However, given that this endpoint is a neurodevelopmental endpoint, it is unclear why the EPA considers the extrapolation in question to be from neonatal animal to adult human, and not (as seems straightforward) from neonatal animal to neonatal human. Therefore, the SAB recommends that the EPA consider application of a $bw^{3/4}$ adjustment as per EPA's 2011 allometric scaling guidance (USEPA, 2011).

With respect to the application of uncertainty factors in derivation of the RfD. The SAB suggests that the EPA further justify the application of an UF of 3 for database deficiency that is based, in part, on the absence of a multi-generational study or extended one generation study (OECD 443 – which is considered a replacement for the multigenerational study). The SAB suggests that the current data base could be considered sufficient as multigenerational studies were conducted and adverse outcomes were demonstrated that are supported by mode of action studies. With the advent of the OECD 443, F1 animals, which have been continually dosed, are only assessed for reproductive effects if triggered (Parental generation are only required to be dosed for 2-weeks prior to mating). Therefore, it is questionable that the OECD 443 will provide any additionally useful reproductive information.

The lack of a study examining functional neurological endpoints following exposure from gestation through lactation was also used as justification for the UF of 3. However, there were 2 oral studies exposing dams at GD 14-17 (McCallister et al. 2008; Sheng et al. 2010), and 2 oral studies exposing

dams or pups directly postnatally (Bouayed et al. 2009; Chen et al. 2012) that evaluated functional endpoints. There were additional gestational exposure studies evaluating receptor gene expression, although there were no studies that examined both gestational and lactational exposure. The EPA should address the question of whether the absence of such a study warrants an additional UF of 3 given that the UF of 10 for inter-individual differences is already included. As part of this deliberation, EPA might also consider whether an EPA developmental neurotoxicity testing (DNT) guideline study and/or extended 1-gen study with a DNT cohort is likely to result in a NOAEL below that of Chen et al. (2012).

Regarding the discussion of uncertainty factors, the SAB suggests that the presentation of the UFs in the assessment be reordered to start with LOAEL-NOAEL… and end with sensitive human, as this is the logical flow when beginning with a POD from an animal study.

The SAB identified two additional issues with the derivation of the RfD. Given the reproductive, developmental and trans-placental effects of BaP, the SAB encourages the EPA to ensure that multi-generational and one-generational effects are addressed, to the extent that data are available. When possible, EPA should identify the sensitive sex in a given study and use the sensitive sex for dose-response modeling.

The SAB found the last portion of charge question 3a, (*Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?*) somewhat vague. In section 2.1.5, the assessment notes that the most sensitive endpoint for RfD development is based on "neurobehavioral changes in rats exposed to benzo[a]pyrene during a susceptible lifestage," i.e., rats exposed *in utero*. Thus, this endpoint is a neurodevelopmental endpoint. The assessment notes in section 2.1.5 that while the RfD derived from this endpoint should be applied to the general population, averaging of exposures over a lifetime should take into account that the critical window of exposure for this developmental endpoint can be much shorter than a lifetime exposure. The SAB interprets this portion of the charge question as asking whether the explanation in section 2.1.5 of the applicability of the concept of a critical window of exposure to the RfD (which is intended to be without significant risk during a *lifetime* of exposure) is appropriate and appropriately conveys the relationship of the critical window of exposure to the lifetime exposure framework of the RfD. Given this interpretation, the SAB agrees that section 2.1.5 is appropriate and appropriately conveys this concept.

3.3.2. Inhalation reference concentration for effects other than cancer

Charge Question 3b. The draft assessment proposes an overall reference concentration of 2 x 10^{-6} mg/m^3 based on decreased fetal survival during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.2.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?

The SAB concludes that the RfC value in the assessment is not currently scientifically supportable in light of concerns with the single study and UF values use, as discussed below. Furthermore, given the available database, the EPA should consider that derivation of a scientifically supportable RfC for BaP may not be possible.

While the endpoint and key study selected (Archibong et al. 2002) are appropriate, the RfC is based only upon this one study that has some specific technical deficiencies which decreases the confidence in the resulting data. For example, blood samples were collected from the orbital plexus (without anesthesia). This is a highly stressful procedure that would be expected to have impacted levels of some hormones, thereby potentially confounding the interpretation of responses attributed to BaP. Furthermore, the concurrent control hormone responses are inconsistent when compared across the exposure groups (e.g., progesterone on GD 17).

The rationale for not employing a BMD approach is unclear. Unequal variances and lack of access to the original datasets are not sufficient reason to avoid BMD modeling of the data in the key study. The EPA has fit BMD models to epidemiological data summaries having these same attributes, and the agency should consider those approaches in the current assessment.

Regarding UFs, the RDDR adjustment used with the key study does not account for systemic toxicokinetics. Given the particle size used in the key study (which would result in significant deposition in the upper respiratory tract of rodents compared to the regional deposition pattern in humans for the same size particle), it may not adequately account for interspecies differences in deposition and ultimate regional dosimetry. Thus, the EPA application of a UF of 3 to address residual uncertainty for interspecies extrapolation is too low.

The Archibong et al. (2002) study found effects at all levels of exposure, so the LOAEL obtained may not be the "true" LOAEL for this endpoint. Thus, this study may not be directly applicable to RfC derivation, and given that this was the only study considered, and a NOAEL was not identified, the actual LOAEL might be much lower and may not be appropriately addressed with the use of an uncertainty factor. EPA should consider the studies of Wu et al. (2003) and Archibong et al. (2012). While these two studies are not replicates of the key study, they may be useful in developing a more comprehensive dose-response relationship for BaP and, thus, perhaps increasing confidence in the LOAEL value used.

In the Wu et al. (2003) study, female rats were exposed for 4h/d to 25, 75, and 100 $\mu g/m^3$ of BaP for 10 days from gestation days 11-20. Dams were allowed to litter and pups were subsequently euthanized at various time points. Brains and livers of F1 pups were collected for measurement of BaP metabolites and mRNA expression profiles for AhR and CYP1A1. The most likely apical endpoint appropriate for determining a POD/BMD is birth index. The authors report that the low exposure group (25 $\mu g/m^3$) was not statistically different from the concurrent control (although it is lower), whereas the 75 and 100 $\mu g/m^3$ exposure groups were statistically lower than the concurrent controls. This suggests that 25 $\mu g/m^3$ may be the NOAEL for this endpoint, under the conditions of this study. However, BMD approaches should also be considered (and contrasted to BMD results of the study by Archibong et al. 2002). Nevertheless, this effect on birth-index is consistent with the effects on pup survival and litter size reported by Archibong et al. (2002).

The Archibong et al. (2012) study explored the potential effects of BaP on the rat ovary, including ovarian estrous cyclicity, hormone production, BaP metabolism, and subsequent effects on reproductive outcomes. Female rats were exposed to 50, 75 or 100 $\mu g/m^3$ of BaP for 4h/d for 14 days and then mated with unexposed males. During exposure, the 100 $\mu g/m^3$ exposure concentration group was associated with an increase in cycle length, changes in hormone levels, and aryl hydrocarbon hydrolase activity.

When the exposure period was over and these animals were mated, this exposure group displayed a lower ovulation rate, fewer pups born and decreased pup survival. Given that all the effects occurred at the highest exposure group examined, and were consistent across endpoints, any of these could be considered for BMD analyses. These data suggest that although adult ovary is a target, fetal development (as demonstrated in Archibong et al. 2002 and Wu et al., 2003) is more sensitive to BaP-mediated toxicity under the exposure condition employed.

3.3.3. Oral Slope Factor for Cancer

Charge Question 3c. The draft assessment proposes an oral slope factor of 1 per mg/kg-d based on alimentary tract tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

The SAB concludes that appropriate studies and models were selected for dose-response analysis. However, insufficient justification was provided for selection of the final slope factor solely from the Beland and Culp (1998) mouse study, instead of the slope factor from the Kroese et al. (2001) rat study or an average of the two. The SAB also raised questions regarding the choice of cross-species scaling factors, and secondary analyses and other additions to the report to improve transparency.

Analysis of Carcinogenicity Data (section 2.3.1)

An oral slope factor for cancer was previously developed by EPA in 1992 and posted on the IRIS database. At that time, BaP was classified as a "probable human carcinogen." The previous oral slope factor (7.3 per mg/kg-day) was derived from the geometric mean of four slope factor estimates based on studies of BaP oral carcinogenesis in Sprague-Dawley rats (2 years) and CFW Swiss mice (7 months) from the combined incidence of forestomach, esophageal and laryngeal tumors. In the current assessment, newer oral carcinogenesis studies were available for further refinement of the oral slope factor (now proposed to be 1 per mg/kg-day), including two 2-year oral carcinogenesis bioassays that associated lifetime BaP exposure with multiple tumor sites including: forestomach, liver, oral cavity, jejunum, kidney, auditory canal, skin and mammary gland in male and female Wistar rats (Kroese et al. 2001) and forestomach, esophageal, tongue and larynx tumors in female B6C3F1 mice (Beland and Culp, 1998). The Kroese et al. (2001) and Beland and Culp (1998) studies were selected as the best available for dose-response analysis and extrapolation to lifetime cancer risk following oral exposure to BaP. These studies were conducted in accordance with Good Laboratory Practice (GLP) and showed dose-related trends in most of the tumor sites. Neither of the studies used in the earlier oral slope factor derivation were used for the current derivation.

The SAB finds that the two selected lifetime oral carcinogenesis studies were well done and appropriate for the dose-response modeling used for cancer oral slope factor derivation. However, it is not clear why only one of the studies, the study by Beland and Culp (1998) was ultimately used in the final derivation of the oral slope factor and not both studies where a (weighted or unweighted geometric) mean or median value might have been derived from the different oral slope factors calculated and presented in the assessment. The SAB was concerned about EPA's choice of the single-sex mouse study that produces the largest cancer slope factor instead of some other slope factor that incorporates data from all studies (rats and mice, males and females) previously judged to be of equal quality and relevance. This decision was not clearly supported by the EPA Guidelines for Carcinogen Risk Assessment (USEPA,

2005a), which allows multiple studies to be combined and suggests "choosing a single dataset if it can be justified as most representative of the overall response in humans."

The SAB acknowledges there are advantages and disadvantages of basing the oral slope factor for cancer on a single mouse study that includes only one sex (female) versus basing it on a rat study that includes both sexes; and, statistical bias that results from using extremity as a selection factor (i.e., always choosing the study that produces the largest slope factor). If no biological basis exists for concluding that the mouse study is more representative of human response than the rat study, EPA should consider averaging over both studies (e.g., simple averaging as used in previous oral slope factor derivation, or meta-analytic/Bayesian averaging as recommended in the 2014 NRC Review of IRIS (NRC, 2014). The oral slope factor for cancer presented in the 1992 BaP assessment was based on an average of slope factors from two different studies, an estimation approach that could have been used in this assessment. An approach similar to the one used in the 1992 BaP assessment should be considered

Dose-Response Analysis (section 2.3.2) and Derivation of the Oral Slope Factor (section 2.3.3)

The oral slope factor for cancer is based on dose-response modeling that uses only the multistage-Weibull model. This model incorporates both the time at which death occurs and the dose in estimating the point of departure from which the cancer slope factor is calculated. This model is generally considered appropriate for the available data, although confidence in the final estimates would be increased if the reader were able to compare the multistage-Weibull model estimate to estimates computed by fitting other dose response models to the same data. These other estimates (and associated deficiencies) could be summarized in an appendix along with the model that is finally chosen. For example, Fitzgerald et al. (2004; their Figure 1 excerpted here) evaluated multiple models of tumor risk and illustrated BMD estimates associated with a 5% extra risk ranged between roughly 0.15 and 0.6 BaP dose (mg/kg/day).

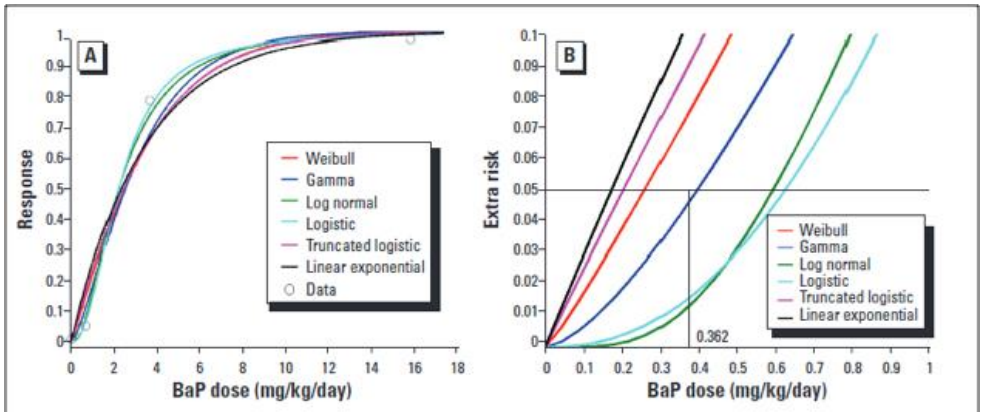

Figure 1. Suite of models fitted to BaP dose–response data (mouse forestomach tumors) reported by Culp et al. (1998). (*A*) MLE fitting of models except the truncated normal, which could not be fitted. (*B*) The extra-risk dose curves of (*A*) in the low-dose region around the 0.05 risk level and averaged dose at 0.362 mg/kg/day.

The adjustments for approximating human equivalent slope factors use the EPA cross-species scaling methodology. Using this approach, time-weighted daily average doses are converted to HEDs on the basis of $BW^{3/4}$ scaling. This allometric scaling is based on current EPA Guidelines (USEPA, 2005a). However, there is uncertainty as to whether this scaling should be applied to all of the tumor sites identified in the two studies. In particular, alimentary tract sites (larynx, esophagus, forestomach) can be

considered portal-of-entry tumor sites, and may not require the application of allometric scaling for these sites. In addition, the BaP oral slope factor calculated in the 1992 BaP assessment used $BW^{2/3}$ for scaling rodent to human HEDs. The impact of this scaling change should be discussed in the assessment. In particular, a comparison of the $BW^{2/3}$ versus $BW^{3/4}$ scaling can easily be accomplished by demonstrating how the scaling change impacts the estimate in the 1992 BaP assessment.

The assessment states that "the oral slope factor should only be used with lifetime human exposures of <0.1 mg/kg-day, because above this level, the dose-response relationship is not expected to be proportional to benzo[a]pyrene exposure" (page 2-30, lines 23-25). How does EPA expect this limitation to be operationalized given that human BaP exposures typically occur within mixtures of PAHs? How often, and in what situations might this condition be invalid?

Uncertainties in the Derivation of the Oral Slope Factor (section 2.3.4)

A number of uncertainties were discussed in the document related to derivation of the oral slope factor for cancer and provided in Table 2-8. Overall, this section was well written. However, The SAB suggests additional discussion in the assessment on two important points.

First, the link between forestomach tumor incidence in mice and rats and cancer incidence in humans is not clearly presented, and the assessment is incomplete without this discussion. The rodent forestomach is highly sensitive to BaP carcinogenesis and represents a major organ for tumor development after oral exposure to this PAH in both rats and mice. The mouse study of Beland and Culp, (1998) is focused almost exclusively on forestomach tumors. The rat study of Kroese et al (2001) provided data on a much broader range of tumor sites. Basing the oral slope factor for cancer on only the mouse study increases the importance of describing the relevance of forestomach tumors in mice to human cancer.

Second, the SAB also raised concerns that the assessment does not discuss how the carcinogenicity of BaP and use of the oral slope factor for cancer are impacted by the fact that humans are exposed to BaP as part of PAH mixtures**.** Some discussion of this issue should be included in the "Uncertainties" section of the assessment. The study by Culp et al. (Carcinogenesis 19:117-124,1998) actually compares the oral carcinogenicity of BaP in a two-year bioassay with two different coal tar mixtures of known content. The coal tar mixtures produce a lower incidence of forestomach tumors compared to BaP, but higher incidence in lung tumors. These data were further evaluated and modeled in the publication by Fitzgerald et al.,2004; their Figure 2 excerpted here). Some discussion and consideration of these data could be provided in more detail.

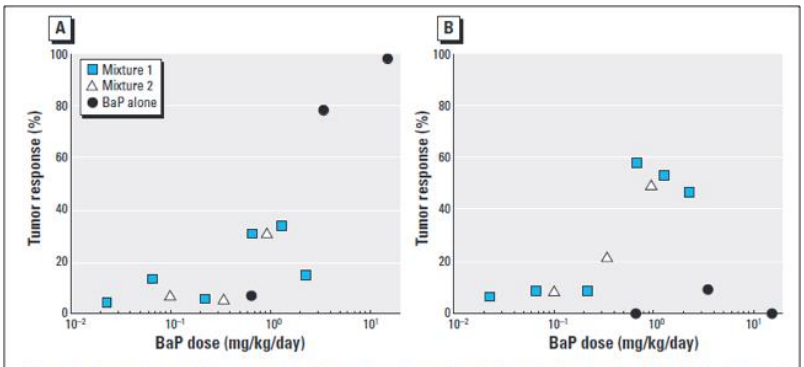

Figure 2. Comparison of dose responses for tumors reported by Culp et al. (1998), plotted for BaP alone and BaP content of coal tar mixtures. (*A*) Forestomach tumors. (*B*) Lung tumors.

Previous IRIS Assessment Oral Slope Factor (section 2.3.5)

A brief description of the derivation of the previous oral slope factor for cancer is given on page 2-32 of the assessment. The SAB suggests that additional discussion comparing the previous analysis with the current analysis might be useful, especially in light of the comments above regarding the use of a single carcinogenicity study for the current slope factor calculation and the differences in scaling between the current and previous slope factor derivation.

3.3.4. Inhalation unit risk for cancer

Charge Question 3d. The draft assessment proposes an inhalation unit risk of 0.6 per mg/m3 based on a combination of several types of benign and malignant tumors in hamsters. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

The SAB concluded that an appropriate study was selected for dose-response analysis and that appropriate models were used to derive the inhalation unit risk (IUR). Although the inhalation unit risk value is scientifically supported, the SAB recommends additional discussion of key assumptions, several sensitivity analyses, and reconsideration of the use of epidemiological data to derive inhalation unit risk values. The SAB also suggests the need for an explicit conclusion statement regarding overall uncertainty of the unit risk value, and a brief discussion of the applicability of this value to typical environmental exposures (especially for sensitive subpopulations).

EPA identified Thyssen et al.(1981) as the only lifetime inhalation cancer bioassay available for describing exposure-response relationships for cancer from inhaled BaP. The experimental design utilized an adult, male hamster model and daily (3-4.5 hr/d) life time exposure to BaP via an inhalation portal of entry (nose-only) for a submicronic sized BaP aerosol. Life-time exposure had average survival durations of 60 to 96 weeks and dose response outcomes included body weight, and incidence and latency of tumors with segmental distributions, i.e., upper respiratory tract (URT), trachea, lung, oro-pharynx, esophagus, and forestomach. EPA relied on this study due to its merits as the "only study of lifetime exposure to inhaled B(a)P." Additional scientific support for Thyssen et al. (1981) arises from a subsequent short communication by the same laboratory (Pauluhn et al. 1985). Although limited in scope, the survival results and presence of neoplastic alterations demonstrate that the experimental design using the hamster model can be replicated for low BaP aerosol concentrations employing an inhalation portal of entry. Overall, the results of Thyssen et al. (1981) found tumors (benign and malignant tumors of the pharynx, larynx, trachea, esophagus, nasal cavity, or forestomach) with increasing BaP concentrations. The SAB identified strengths of the approach (durations of exposure to natural death, histologic exam of tissues, monitoring of exposure concentrations) and limitations (lack of distal lung tumors, variation in exposure concentrations, BaP exposure aerosol was developed using sodium chloride condensation nuclei) and these issues were fully addressed in section 2.4.4 of the assessment.

Due to the merits of a life-time inhalation animal model study that demonstrated carcinogenicity results, EPA's selection of Thyssen et al. (1981) for dose-response assessment is appropriate. Dose-response modeling and unit risk estimation for those data used appropriate methods, and the multistage Weibull model fit was adequate. Although the SAB agrees with EPA that the multistage Weibull model is preferable due to incorporation of time-to-tumor data, the final unit risk value can be further supported by: 1) supplemental sensitivity analyses using other dose-response models; 2) alternative assumptions

about latency and cross-species scaling of doses; and 3) not eliminating from the analysis all animals without confirmed examination of one or more of the pharynx or respiratory tract tissues. The panel also recommends additional discussion of the assumptions used to derive the unit risk (that "any metabolism of benzo(a)pyrene is directly proportional to breathing rate and that the deposition rate is equal between species" on p. 2-35, lines 6-8, and selection of body weight scaling factors in relation to "portal of entry," as discussed in the EPA Guidelines for Carcinogen Risk Assessment). EPA should also state a conclusion regarding overall uncertainty or level of confidence for the IUR, as endorsed on p. 118 of the NRC 2014 review of the IRIS program (NRC, 2014).

Given the extensive human studies of lung cancer with airborne inhalation exposures to PAHs by coke oven, and aluminum smelter workers (i.e., Table 1-11, summary of Tier 1 epidemiologic based reports of BaP in relation to lung cancer, pp. 1-55 to 1-56, and specifically, reports by Armstrong and Gibbs (2009); Spinelli et al.(2006); Xu et al. (1996); and Gibbs and Labreche (2014), the SAB recommends that EPA give further consideration to selection of occupational studies (or meta-analysis of occupational studies) to develop unit risk estimate(s) for inclusion in Table 2-9. Although interpretation of the epidemiological evidence is challenging given that exposures were to mixtures of PAHs with poorly understood interactions, a model using relative potency factors and an assumption of dose additivity was reasonably accurate for some PAH mixtures and conservative for others in one investigation (U.S. EPA, 1990), and should be considered for adjustment of epidemiological results in estimation of the unit risk attributable to BaP alone. Uncertainty and risk of bias due to exposure measurement error, healthy worker effects, habituation, and/or co-exposure to cigarette smoke products should also be considered and weighed against uncertainties regarding cross-species extrapolation of the unit risk from hamsters to humans.

It may be helpful for EPA to address how reasonable it is that lifetime exposures will be in the approximately linear low dose region where the unit risk is applicable (<0.3 mg/m^3, the human equivalent POD). The SAB recognizes that a nationwide BaP exposure assessment is far beyond the scope of the assessment, but reference to typical exposure ranges may be helpful to readers.

3.3.5. Dermal Slope factor for cancer

Charge Question 3e. The draft assessment proposes a dermal slope factor of 0.006 per µg/day based on skin tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and scaling from mice to humans? *Does the method for cross-species scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?*

Neither the proposed dermal slope factor nor the proposed method for cross-species scaling is sufficiently scientifically supported. Discussion is provided below that explains the SAB's concerns with the justifications of these two analyses in the assessment.

Analysis of carcinogenicity data (choice of Studies) (section 2.5.1)

In the choice of skin cancer bioassay studies for developing the dermal slope factor (DSF), the BaP assessment reviewed 10 complete carcinogenicity mouse skin tumor bioassay studies from 1959 to 1997 (summarized in Table 2-11) and the Sivak et al. (1997) study was chosen as the principal study. Other skin cancer bioassay studies are mentioned and excluded for further analysis because: (1) only one BaP level was considered; (2) all dose levels induced 90-100% incidence of tumors; (3) dose applications

were once/week (Nesnow et al. 1983) or once/2 weeks (Levin et al., 1977); and (4) dose was delivered in a vehicle that interacted or enhanced BaP carcinogenicity.

Recommendation:

- EPA should consider adding Nesnow et al. (1983) and Levin et al. (1977) studies to Table 2-11 and should consider combining results from the different studies shown in Table 2-11. This would strengthen the derived DSF. Skin cancer bioassay studies that examined only one BaP level or observed 90-100% incidence of tumors are not suitable for estimating points of departure (POD). However, consistencies in the observations of these studies with observations from the studies listed in Table 2-11 and those used to develop the POD and DSF would strengthen the derived DSF.

The EPA review of the epidemiologic evidence of skin cancer in humans is not sufficiently thorough. The assessment cites evidence of an excess of skin cancer in studies of roofers (Hammond et al. 1976) and workers exposed to creosote-treated wood (Karlehagen et al., 1992; Tornqvist, 1986), but these groups work outside and would thus have substantial exposure to UV. The assessment also notes that recent studies of chimney sweeps do not demonstrate an increased skin cancer risk (Hogstedt et al. 2013). The assessment does not cite or discuss some older studies that reported an excess of skin cancer in destructive distillation of coal, shale oil extraction, and workers exposed to creosote in brick making and wood impregnation (Boffetta et al. 1997).

Recommendation:

- The EPA should more thoroughly review the evidence for skin cancer in studies of coke, steel and iron, coal gasification and aluminum workers given their relevance for evaluating the appropriateness of using the mouse based risk assessment model for predicting skin cancer risk in humans.

The SAB notes that epidemiologic studies of therapeutic use of coal tar preparations do not provide an adequate basis for either hazard identification or the derivation of a dermal slope factor due to uncertainties regarding the PAH dose and the relevance of the (psoriasis patients) population.

Dose-response analysis (section 2.5.2) and Derivation of the dermal slope factor (section 2.5.3.)

The BaP assessment states that mass rather than mass/area can be used as the appropriate dose metric for cancer risk at "low doses" of BaP. The SAB notes that published dermal slope factors for BaP skin carcinogenesis have used mass and mass/skin area as dose metrics and there does not appear to be any empirical data available to inform a choice between these two dose metrics or to select another.

Experimental studies have demonstrated that equal masses of chemical absorb into the skin when the area of direct chemical contact is less than the applied skin area (i.e., the mass of chemical applied is too small to completely cover the application area). For example, Roy and Singh (2001) reported that the percentage of BaP applied on contaminated soil that was absorbed was independent of the mass of soil applied until the skin surface area was completely covered with soil; further increases in the mass of soil applied caused the percent BaP absorption to decrease. The DSF derived from the skin cancer bioassay in mice is based on the applied dose, which most probably closely approximates the absorbed dose. The time between dose applications was long enough and the applied doses small enough in the mouse studies for approximately 100% absorption. For example, Wester et al. (1990) observed 51% absorption

in vivo in monkey and 24% absorption *in vitro* in humans for 0.5 µg/cm^2 in 24 h. The absorption rates through mouse skin are faster than through humans and monkeys. The conclusion that absorbed dose approximately equals the applied dose assumes that dose losses were minimal; therefore, study protocols in the document should be evaluated for factors that may have affected losses of the applied dose (e.g.. by grooming).

Recommendations:

- The SAB does not have a specific recommendation as to dose metric, but strongly recommends that in the absence of empirical data the decision be based upon a clearly articulated, logical, scientific structure that includes what is known about the dermal absorption of BaP under both conditions of the bioassay(s) and anticipated human exposures, as well as the mechanism of skin carcinogenesis of BaP.
- The choice of dose metric needs to be better justified and EPA should provide a convincing argument for the use of mass as the dose metric.
- The SAB recommends that cancer risk calculated from the derived DSF should use **absorbed dose** and not exposed applied dose.
- EPA should describe what constitutes a "low dose" for the assumption that mass of BaP is the appropriate dose metric for calculating the DSF from the skin cancer bioassay studies and for estimating cancer risk in humans. This should be consistent with the proposed logical structure for skin cancer from skin exposure to BaP, which is a solid at skin temperature. Issues to consider include:
    - For dermal absorption, the skin area with direct chemical contact must be less than the total applied area; i.e., mass of BaP applied cannot completely cover the applied area. For BaP deposited onto skin from a volatile solvent, the mass of BaP that would give a theoretical uniformly thick film <1 µm (i. e., ~135 µg of BaP/cm2) would be too small to completely cover the application area, where: Theoretical thickness of a uniform film on the application area = [(BaP mass applied)/(application area)]/$\boldsymbol{\rho}_{BaP;}$ $\boldsymbol{\rho}_{BaP}$= density of BaP= 1.35 g/mL.
    - Metabolism in the target tissue (the viable epidermis) should not be saturated. The document identifies the linear limit for using the slope factor to calculate cancer risk in humans based on the human equivalent point-of-departure (POD_{HED} = 17.9 µg/day) estimated from the mouse POD_M adjusted by the mouse-to-human scaling factor as the $BW^{¾}$. This is an appropriate limit that could be smaller than 17.9 µg/day for different scaling factor approaches.
- EPA should consider adding diagrams illustrating the logical structure (physiological steps to carcinogenesis) to facilitate choices of dose metric and cross-species scaling
- EPA should consider adding diagrams illustrating the steps involved in calculating human cancer risk based on skin cancer bioassay studies in mice; for example
    - Tumors observed in mouse studied as a function of time and exposed dose
    - Exposed dose ≈ applied dose to estimate in mice: PODm and DSFm
    - DSFm scaled to the human DSFh
    - Estimate of absorbed dose from exposed dose and exposure scenario
    - Human cancer risk = DSFh x (Absorbed dose)

Dermal slope factor cross-species scaling

According to the assessment, the starting point is the dermal slope factor in the mouse (i.e., DSFm= 1.7 (µg/day)$^{-1}$), which is adjusted by the appropriate human to mouse ratio to obtain the dermal slope factor

in humans (DSFh). Experimental cancer risk information for scaling from mouse to human skin cancer from dermal exposure is not available. It is unknown if the chosen approach for scaling of skin cancer risk from BaP exposure to skin is similar to interspecies differences in whole body toxicokinetics, which is the approach (i.e., allometric scaling using $BW^{3/4}$) adopted by EPA. The assessment lists alternative approaches for scaling. The science for choosing the best approach is uncertain.

Recommendations:

- The chosen scaling approach should be supported by a coherent logical structure. Differences between mouse and human skin should be considered in light of the proposed logical structure for skin cancer risk; for example:
  - Thickness of and metabolic rates in the target tissue (i.e., the viable epidermis layer).
  - Differences in stratum corneum thickness will affect the absorbed dose from a given exposed dose applied to humans compared with mice. However, it may not affect the cross-species scaling of the DSF, which is based on absorbed dose.

Uncertainties in the derivation of the dermal slope factor

The cross-species mouse-to-human scaling of the DSF is a significant contributor to uncertainties.

Other recommendations for describing cancer risk calculated with the DSF

- The cancer risk calculation in mice (and therefore in humans) depends on absorbed dose; i.e., Cancer Risk = DSF x (Absorbed dose). EPA should state clearly how the absorbed dose estimates from exposed dose enters the calculation of cancer risk.
- In actual BaP exposures (from soil or other environmental media), the absorbed dose should be estimated from the exposed dose and the exposure scenario.
- A soil-to-acetone absorption ratio as described in the response to public comments is unnecessary.
- Cancer risk from BaP in soil should be calculated from the estimated absorbed dose from exposure to BaP contaminated soil.
- Examples of cancer risk estimates from exposure to BaP contaminated soil will use an estimate of the absorbed dose taken from the literature (or RAGS, Vol. 1, Part E). Because the assessment does not critically review this literature,
  - The literature of dermal absorption measurements from BaP contaminated soils should be listed; and
  - The estimate of absorption used in the risk calculation should be identified as an example (and not an endorsement of the value used).
- Each environmental media will have its own absorption characteristics that should be considered in estimating an absorbed dose for estimating cancer risk.

3.3.6. Age-dependent adjustment factors for Cancer

Charge Question 3f. The draft assessment proposes the application of age-dependent adjustment factors based on a determination that benzo(a)pyrene induces cancer through a mutagenic mode of action. Do the available mechanistic studies in humans and animals support a mutagenic mode of action for cancer induced by benzo(a)pyrene?

The available mechanistic studies in humans and animals support a mutagenic mode of action for BaP-induced cancers. Given that the EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposures to Carcinogens* (U.S. EPA, 2005b) establishes a rational approach for the adjustment of tumor risk for exposures at different ages to carcinogens with a mutagenic mode of action, the SAB concludes that the proposed use of age-dependent adjustment factors (ADAFs) is justified.

3.4. Executive Summary

Charge Question 4. Does the executive summary clearly and appropriately present the major conclusions of the assessment?

The SAB found that the major conclusions of the assessment were clearly and appropriately presented in the Executive Summary. Changes made to the body of the assessment in response to the SAB recommendations that impact the derivation of the chronic RfD/RfC or cancer slope factors should be incorporated into the Executive Summary. In addition, the SAB had a number of suggestions for improvement of the Executive Summary:

- The purpose of the gray box text at the beginning of the Executive Summary is not immediately apparent. During the SAB panel meeting, the agency clarified that this box is intended to be a lay language abstract for the report. That means that it has a different audience than the rest of the document, and the SAB suggests that it stand alone from the Executive Summary and be clearly identified as a lay language abstract or summary. The SAB further suggests that the gray box text be examined to insure that the health literacy level is commensurate with the lay public as target audience.

- For audiences that will focus on the Executive Summary, it is not clear in the narrative presented why a toxicological review focusing on BaP is relevant. The SAB suggests adding introductory text to the Executive Summary explaining the public health relevance of the assessment especially related to the importance of evaluating hazard and risk from human exposures to PAHs present in PAH mixtures.

- Although the SAB has no specific advice regarding the appropriate length for the Executive Summary, the agency should strive to capture the important conclusions in a summary that is of readable length.

- The basis upon which levels of confidence in toxicity values (i.e., "low," "medium," or "high") are reached is not always apparent, and therefore the meaning of these descriptors as presented in the Executive Summary will be unclear. The SAB suggests adding a few sentences in the Executive Summary to explain how confidence levels are determined.

3.5. Public Comments

Charge Question 5. In August 2013, EPA asked for public comments on an earlier draft of this assessment. Appendix G summarizes the public comments and this assessment's responses to them. Please comment on EPA's responses to the scientific issues raised in the public comments. Please consider in your review whether there are scientific issues that were raised by the public as described in Appendix G that may not have been adequately addressed by EPA.

The SAB found that most of the scientific issues raised by the public, as described in Appendix G, were adequately addressed by EPA.[1] However, there were some issues that the SAB requests additional clarification from EPA. These issues are identified below with reference to relevant sections of the SAB report.

- *Comment: Metric used to characterize results in the elevated plus maze* (pg G-5). Public commenters noted that the way the maze response was quantified is not the preferred way. The EPA response agrees with the point raised, but explains that data necessary to quantify response in the preferred way were not available, but there was enough information available to conclude that the results presented are valid (i.e., were not unduly influenced by changes in general locomotor or exploratory behaviors. The SAB's discussion regarding these results is summarized in the response to Charge Question 2a.

- *Comment: Use of decreased anxiety-like effects as a critical effect (p. G-6).* Public commenters questioned whether decreased anxiety-like effects are adverse effects. The EPA response explains that decreased anxiety represents a clear change in nervous system function and can impair an organism's ability to react to a potentially harmful situation. Further discussion on this endpoint is provided in the response to Charge Question 2a.

- *Comment: Cross-species extrapolation of dermal slope factor (p. G-11).* Public commenters stated that differences between mouse and human skin should be accounted for in cross-species extrapolation. The EPA response notes that biological information is not currently sufficient to develop robust models for cross-species extrapolation, and states that allometric scaling using body weight to the ¾ power was selected based upon observed differences in the rates of dermal absorption and metabolism of benzo(a)pyrene. The SAB found that this cross-species scaling factor was not sufficiently justified, as discussed in the response to Charge Question CQ3e.

- *Comment: Uncertainties regarding implementation of the dermal slope factor* (p. G-12). Two aspects of the public comments under this topic received significant discussion by the Panel. One is a comment that a 13% dermal absorption factor for benzo(a)pyrene may not be appropriate. The EPA response explains the origin of the value, but acknowledges that it may be a high estimate. The SAB also has concerns about the dermal absorption value, as discussed in the response to Charge Question 3e. The SAB provided specific suggestions. The second comment is that the dose metric of µg/d is not appropriate for the slope factor in view of the mode of action. The EPA response is that dermal bioassays report total dose applied to the skin but do not quantify the area over which the dose is applied. The SAB concluded that the dose metric has not been sufficiently justified by EPA, as explained in the response to Charge Question 3e.

- *Comment: "Real world" validation of dermal slope factor* (p. G12). Public commenters recommended that EPA perform calculations of risk from dermal exposure to PAHs using the

[1] Tthe Draft Toxicological Review for Benzo[a]pyrene that the SAB was asked to review contained only those public comments received by EPA prior to the completion of the document (i.e., responses EPA received on the 2013, draft). Thus, the SAB's comments in response to this charge question relate to t EPA's responses to those earlier public comments.

proposed dermal slope factor to determine whether the value is scientifically supportable. Commenters discussed that this type of calculation shows skin cancer risks from common PAH exposures such as the use of pharmaceutical coal tar products that are unrealistically high. In their response, EPA indicated that sufficient details were not provided to allow EPA to reproduce the calculations performed by the public commenters, and provided their own estimate of risk from exposure to benzo(a)pyrene in soil showing a low excess cancer risk (6×10^{-6} for average lifetime exposure that occurs during childhood and 1×10^{-6} for average lifetime exposure that occurs during adulthood).

With respect to the dermal cancer slope factor, the SAB supports the application of a "fidelity exercise" for proposed toxicity values to determine whether the toxicity values yield plausible upper bound risk estimates. Generally, this exercise consists of using the proposed toxicity value to estimate risk from one or more exposure scenarios and determine whether the results exceed lifetime risk estimates derived from actual disease incidence (Howlader 2015) for the adverse effect(s) of interest. The SAB finds limitations in the fidelity exercise approaches taken by both the public commenters and the EPA in its response. For example, the EPA estimation of cancer risk from benzo(a)pyrene alone does not reflect actual circumstances of exposure, which almost always occurs as a mixture of carcinogenic PAHs (benzo(a)pyrene plus others of varying potency). On the other hand, the limitations of coal tar therapeutics studies make them largely uninformative with regard to the question of whether BaP induces skin cancer in humans. The public commenter's use of upper percentile exposure values to represent exposure of the overall population tends to exaggerate risk, and the recognized under-reporting of skin cancer[2] was not taken into account in comparisons. Further, the inherent conservative nature of toxicity values should be recognized and taken into consideration in such analyses. The SAB suggests an improved fidelity exercise to address concerns that the proposed dermal cancer slope factor may lead to unrealistic cancer risk estimates.

As a general comment, the SAB supports the approach taken by EPA in creating Appendix G in which the most important scientific issues presented by public commenters are captured and arranged by topic, with reference to the public commenters raising the issue. A more extensive approach, such as providing comment-by-comment responses would be inefficient and cumbersome in a toxicological review. The SAB is aware of contention by some public commenters that their comments were not adequately captured and articulated in Appendix G. To minimize such concerns in future toxicological reviews, the SAB urges the EPA to provide greater transparency in how public comments are distilled into a list of scientific issues meriting an EPA response in the assessment. In particular, the SAB suggests that EPA provide a short description of the process of deciding which comments to include in a public response appendix and how comments are aggregated within the appendix. In particular, it would be helpful if EPA provided a table within the assessment showing the topics under which comments are aggregated, which commenters provided comments within each topic, and the dates on which the comments were made.

[2] ACS, 2015, American Cancer Society, Cancer Facts & figures 2015. Atlanta: American Cancer Society; 2015. p 21. "Skin cancer is the most commonly diagnosed cancer in the United States. However, the actual number of the most common types – basal cell and squamous cell skin cancer (i.e., keratinocyte carcinoma), more commonly referred to as nonmelanoma skin cancer (NMSC) – is very difficult to estimate because these cases are not required to be reported to cancer registries. The most recent study of NMSC occurrence estimated that in 2006, 3.5 million cases were diagnosed among 2.2 million people. NMSC is usually highly curable."

REFERENCES

Aboutabl ME, Zordoky BN, El-Kadi AO. (2009). 3-Methylcholanthrene and benzo(*a*)pyrene modulate cardiac cytochrome P450 gene expression and arachidonic acid metabolism in male Sprague Dawley rats . *Br J Pharmacol* 158 , 1808 – 19.

Aboutabl ME, Zordoky BN , Hammock BD , El-Kadi AO . (2011). Inhibition of soluble epoxide hydrolase confers cardioprotection and prevents cardiac cytochrome P450 induction by BaP. *J Cardiovasc Pharmacol* 57, 273– 81.

Alejandro, N. F., Parrish, A. R., Bowes III, R.C., Burghardt, R.C. and Ramos, K. S. Phenotypic profiles of cultural glomerular cells following repeated cycles of hydrocarbon injury. *Kidney International* 57(4), 1571-1580, Apr 2000. PMID: 10760092.

Alexandrov, K; Rojas, M; Geneste, O., et al. (1992). An improved fluorometric assay for dosimetry of benzo(a)pyrene diol-epoxide-DNA adducts in smokers' lung: comparisons with total bulky adducts and aryl hydrocarbon hydroxylase activity. *Cancer Res* 52: 6248-6253.

Archibong, AE; Inyang, F; Ramesh, A; Greenwood, M; Nayyar, T; Kopsombut, P; Hood, DB; Nyanda, AM. (2002). Alteration of pregnancy related hormones and fetal survival in F-344 rats exposed by inhalation to benzo(a)pyrene. *Reproductive Toxicolgy* 16:801-808.

Archibong, AE; Ramesh, A; Niaz, MS; Brooks, CM; Roberson, SI; Lunstra, DD. (2008). Effects of benzo(a)pyrene on intra-testicular function in F-344 rats. *Int J Environ Res Public Health* 5: 32-40.

Archibong, AE; Ramesh, A; Inyang, F; Niaz, MS; Hood, DB; Kopsombut, P. (2012). Endocrine disruptive actions of inhaled benzo(a)pyrene on ovarian function and fetal survical in fisher F-344 adult rats. *Reproductive Toxicology* 34:635-643.

Armstrong, BG; Gibbs, G. (2009) Exposure-response relationship between lung cancer and polycyclic aromatic hydrocarbons (PAHs). *Occup Environ Med* 66:740–746.

Armstrong, B; Hutchinson, E; Unwin, J; Fletcher, T. (2004). Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: a review and meta-analysis. *Environ Health Perspect* 112(9):970-8.

Behrens, T; Schill, W; Ahrens, W. (2009). Elevated cancer mortality in a german cohort of bitumen workers: extended follow-up through 2004. *J Occup Environ Hyg* 6:555–561.

Beck CR, Garcia-Perez JL, Badge RM, Moran JV. 2011. LINE-1 Elements in Structural Variation and Disease. *Annual Review of Genomics and Human Genetics* 12: 187 -215

Beland, F; Culp, S. (1998). Chronic bioassay of two composite samples from selected manufactured gas plant waste sites [unpublished report]. (Technical Report 6722.02). Jefferson, AK; National Center for Toxicological Research.Bhate, SM; Sharpe, GR; Marks, JM; Shuster, S; Ross, WM. (1993). Prevalence of skin and other cancers in patients with psoriasis. *Clinical and Experimental Dermatology* 18: 401-404.

Boffetta, P; Jourenkova, N; Gustavsson, P. (1997). Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. *Cancer Causes Control* 8:444-472.

Borman, S.M., P.J. Christian, I.G. Sipes, and P.B. Hoyer. 2000. Ovotoxicity in female Fischer rats and B6 mice induced by low-dose exposure to three polycyclic aromatic hydrocarbons: comparison through calculation of an ovotoxic index. *Toxicol Appl Pharmacol*. 167:191-198.

Bosetti, C; Boffetta, P; La Vecchia, C. (2007). Occupational exposures to polycyclic aromatic hydrocarbons, and respiratory and urinary tract cancers: a quantitative review to 2005. *Ann Oncol* 18(3):431-46.

Bostrom, CE; Gerde, P; Hanberg, A; Jernstrom, B; Johansson, C; Kyrklund, T; Rannug, A; Tornqvist, M; Victorin, K; Westerholm, R. (2002). Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ Health Perspect* 110:451-488.

Bouayed J, Desor F, Rammal H, Kiemer AK, Tybl E, Schroeder H, Rychen G, Soulimani R (2009) Effects of lactational exposure to benzo[alpha]pyrene (B[alpha]P) on postnatal neurodevelopment, neuronal receptor gene expression and behaviour in mice. *Toxicology* 259:97-106.

Bouayed, J; Bohn, T; Tybl, E; Kiemer, AK; Soulimani, R. (2012). Benzo[α]pyrene-induced anti-depressive-like behavior in adult female mice: role of monoaminergic systems. *Basic & Clinical Pharmacology & Toxicology Online Pharmacology Online* 110: 544-550.

Boysen, G; Hecht, SS. (2003) Analysis of DNA and protein adducts of benzo[a]pyrene in human tissues using structure-specific methods. *Mutation Res* 543:17-30.

Brown LA, Khousbouei H, Goodwin JS, Irvin-Wilson CV, Ramesh A, Sheng L, McCallister MM, Jiang GC, Aschner M, Hood DB (2007) Down-regulation of early ionotrophic glutamate receptor subunit developmental expression as a mechanism for observed plasticity deficits following gestational exposure to benzo(a)pyrene. *Neurotoxicology* 28:965-978.

Burchiel, SW, Lauer, FT, Dunaway, SL, Zawadzki, J, McDonald, JD, and Reed, MD. (2005) Hardwood smoke alters murine splenic T cell responses to mitogens following a six month whole body inhalation exposure. *Toxicol Appl Pharmacol*, 202: 229-236.

Burchiel, SW, and Luster, MI (2001) Signaling by environmental polycyclic aromatic hydrocarbons in human lymphocytes. *Clin Immunol* 98: 2-10.

Burdick, AD; Davis, JD; Liu, KJ; Hudson, LG; Shi, H; Monske, ML; Burchiel, SW. (2003) Benzo[a]pyrene quinones increase cell proliferation, generate reactive oxygen species, and transactivate the epidermal growth factor receptor. *Cancer Research* 63:7825-7833.

Burstyn, I; Kromhout, H; Partanen, T; Svane, O; Langard, S; Ahrens, W; Kauppinen, T; Stucker, I; Shaham, J; Heederik, D; Ferro, G; Heikkila, P: Hooiveld, M; Johansen, C; Randem, BG; Boffetta, P. (2005). Polycyclic aromatic hydrocarbons and fatal ischemic heart disease. *Epidemiology* 16: 744-750.

Burstyn, I; Kromhout, H; Johansen, C; Langard, S; Kauppinen, T; Shaham, J; Ferro, G; Boffetta, P. (2007). Bladder cancer incidence and exposure to polycyclic aromatic hydrocarbons among asphalt pavers. *Occup Environ Med* 64: 520-526.

Chen, X; An, H; Ao, L; Sun, L; Liu, W; Zhou, Z; Wang, Y; Cao, J. (2011). The combined toxicity of dibutyl phthalate and benzo[a]pyrene on the reproductive system of male Sprague Dawley rats in vivo. *J. Hazardous Materials* 186: 835-841.

Chen C, Tang Y, Jiang X, Qi Y, Cheng S, Qiu C, Peng B, Tu B (2012) Early postnatal benzo(a)pyrene exposure in Sprague-Dawley rats causes persistent neurobehavioral impairments that emerge postnatally and continue into adolescence and adulthood. *Toxicol Sci* 125:248-261.

Chiazze, L; Watkins, DK; Amsel, J. (1991) Asphalt and risk of cancer in man [Review]. *Br. J Ind Med* 48: 538-542.

Chung, J.Y., Y.J. Kim, J.Y. Kim, S.G. Lee, J.E. Park, W.R. Kim, Y.D. Yoon, K.S. Yoo, Y.H. Yoo, and J.M. Kim. 2011. Benzo a pyrene Reduces Testosterone Production in Rat Leydig Cells via a Direct Disturbance of Testicular Steroidogenic Machinery. *Environmental Health Perspectives*. 119:1569-1574.

Culp, SJ; Beland, FA. (1994) Comparison of DNA adduct formation in mice fed coal tar or benzo[a]pyrene. *Carcinogenesis* 15:247-252.

Culp, SJ; Gaylor, DW; Sheldon, WG; Goldstein, LS; Beland, FA. (1998). A comparison of the tumors induced by coal tar and benzo[a]pyrene in a 2 year bioassay. *Carcinogenesis* 19:117-124.

Daudel, P; Duquesne, M; Vigny, P; Grover, PL; Sims, P. (1975). Fluorescence spectral evidence that benzo[a]pyrene-DNA products in mouse skin arise from diol-epoxides. *FEBS Letters* 57:250-253.

Davila, DR, Romero, DL, and Burchiel, SW (1996) Human T cells are highly sensitive to suppression of mitogenesis by polycyclic aromatic hydrocarbons and this effect is differentially reversed by α-naphthoflavone, *Toxicol Appl Pharmacol* 139: 333-341.

Dean JH, Luster, MI, Boorman, GA, Lauer, LD, Leubke, R, Lawson, L (1983). Selective immunosuppression resulting from exposure to the carcinogenic congener of benzopyrene in B6C3F1 mice. *Clin. Exp. Immunol*. 52: 199-206.

De Jong, WH; Kroese, ED; Vos, JG; Van Loveren, H. (1999). Detection of immunotoxicity of 1 benzo[a]pyrene in a subacute toxicity study after oral exposure in rats. Toxicol Sci 50: 214-220. http://dx.doi.org/10.1093/toxsci/50.2.21.

Dejmek J, Solanský I, Benes I, Lenícek J, Srám RJ. (2000) The impact of polycyclic aromatic hydrocarbons and fine particles on pregnancy outcome. *Environ Health Perspect.* 2000 Dec;108(12):1159-64.

Dietert, RR, Etzel, RA, Chen, D, Halonen, M, Holladay, SD, Jarabek, AM, Landreth, K, Peden, DB, Pinkerton, K, Smialowicz, RJ, Zoetis, T. (2000). Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary [Review]. *Environ Health Perspect* 108 Suppl 3: 483-490. 23

Dietert, RR, Piepenbrink, MS. (2006). Perinatal immunotoxicity: Why adult exposure assessment fails to predict risk [Review]. Environ Health Perspect 114: 477-483. http://dx.doi.org/10.1289/ehp.8566 26

Duarte-Salles, T; Mendez, MA; Meltzer, HM; Alexander, J; Haugen, M. (2013). Dietary benzo[a]pyrene intake during pregnancy and birth weight: associations modified by vitamin C intakes in the Norwegian mother and child cohort study. *Environment International* 60: 217-223.

Duarte-Salles, T; Mendez, MA; Morales, E; Bustamante, M; Rodriguez-Vicente, A; Kogevinas, M; Sunyer, J. (2012). Dietary benzo[a]pyrene and fetal growth: Effect modification by vitamin C intake and glutathione S-transferase P1 polymorphism. *Environment international* 45, 1-8.

Duarte-Salles, T; Mendez, MA; Pessoa, V; Guxens, M; Aguilera, I; Kogevinas, M; and Sunyer, J. (2010) Smoking during pregnancy is associated with higher dietary intake of polycyclic aromatic hydrocarbons and poor diet quality. *Public Health Nutritrition* 13: 2034-2043.

Einaudi, L., B. Courbiere, V. Tassistro, C. Prevot, I. Sari-Minodier, T. Orsiere, and J. Perrin. 2014. In vivo exposure to benzo(a) pyrene induces significant DNA damage in mouse oocytes and cumulus cells. *Human Reproduction*. 29:548-554.

Fitzgerald, DJ; Robinson, NI; Pester, BA. (2004). Application of benzo(a)pyrene and coal tar tumor dose-response data to a modified benchmark dose method of guideline development. *Environmental Health Perspectives* 112: 1341-1346.

Friesen, MC; Demers, PA; Spinelli, JJ; Eisen, EA; Lorenzi, MF; Le, ND.(2010). Chronic and acute effects of coal tar pitch exposure and cardiopulmonary mortality among aluminum smelter workers. *Am J Epidemiol* 172: 790-799.

Gan, TR; Xiao, SP; Jiang, Y; Hu, H; Wu, YH; Duerksen-Hughes, PJ; Sheng, JZ; and Yang, J. (2012). Effects of Benzo[a]pyrene on the contractile function of the thoracic aorta of Sprague-Dawley rats. *Biomed Environ Sci* 25:549-56

Gao, M., Y. Li, S. Sun, W. Shah, S. Yang, Y. Wang., and J. Long 2011. Benzo[a]pyrene exposure increases toxic biomarkers and morphological disorders in mouse cervix. *Basic & Clinical Pharmacology & Toxicology*, 109, 398–406

Gibbs, GW; Sevigny M (2007a) Mortality and cancer experience of Quebec aluminum reduction plant workers, part 4: cancer incidence. *J Occup Environ Med* 49:1351–1366.

Gibbs, GW; Sevigny, M. (2007b) Mortality and cancer experience of Quebec aluminum reduction plant workers. Part 3: monitoring the mortality of workers first employed after January 1, 1950. *J Occup Environ Med* 49:1269–1287.

Gibbs, GW; Labrèche, F. (2014) Cancer risks in aluminum reduction plant workers: a review. *JOEM*, 56: S40-S48.

Grova, N; Valley, A; Turner, JD; Morel, A; Muller, CP; Schroeder, H. (2007). Modulation of behavior and NMDA-R1 gene mRNA expression in adult female mice after sub-acute administration of benzo(a)pyrene. *Neurotoxicology* 28:630-636.

Grova, N; Schroeder, H; Farinelle, S; Prodhomme, E; Valley, A; Muller, CP.(2008). Sub-acute administration of benzo[a]pyrene reduces anxiety-related behavior in adult mice and modulates regional expression of N-methyl-D-aspartate (NMDA) receptors genes in relevant brain regions. *Chemosphere* 73:S295-S302.

Grover, PL; Hewer, A; Pal, K; Sims, P. (1976). The involvement of a diol-epoxide in the metabolic activation of benzo[a]pyrene in human bronchial mucosa and in mouse skin, *Int. J. Cancer* 18:1-6.

Gunter, MJ; Divi, RL; Kulldorff, M; Vermeulen, R; Haverkos, KJ; Kuo, MM; Strickland, P; Poirier, MC; Rothman, N; Sinha, R. (2007). Leukocyte polycyclic aromatic hydrocarbon-DNA adduct formation and colorectal adenoma. *Carcinogenesis* 28(7):1426-1429. HERO ID1011897

Hammond, EC; Selikoff, IJ; Lawther, PL; Seidman, H. (1976). Inhalation of benzo[a]pyrene and cancer in man. *Ann N Y Acad Sci* 271: 116-124.

Hannuksela-Svahn, A; Pukkala, E; Laara, E; Poikolainen, K; Karvonen, J. (2000). *Psoriasis, its treatment, and cancer in a cohort of Finnish patients. J of Investigative Dermatology* 114: 587-590.

Hansen, ES. (1989). Cancer incidence in an occupational cohort exposed to bitumen fumes. *Scand J Work Environ Health* 15: 101-105.

Hansen, ES. (1991). Mortality of mastic asphalt workers. *Scand J Work Environ Health* 17: 20-24.

Hardin, JA, Hinoshita, F, Sherr, DH. (1992). Mechanisms by which benzo[a]pyrene, an environmental 23 carcinogen, suppresses B cell lymphopoiesis. *Toxicol Appl Pharmacol* 117: 155-164.

Health Canada (2015) Draft "Benzo[a]pyrene in Drinking Water" at: http://www.hc-sc.gc.ca/ewh-semt/consult/_2015/bap/draft-ebauche-eng.php

Holladay, SD; Smith, BJ. (1994). Fetal hematopoietic alterations after maternal exposure to benzo[a]pyrene: A cytometric evaluation. *J Toxicol Environ Health* 42: 259-273.

Holladay, SD; Smith, BJ. (1995). Benzo[a]pyrene-induced alterations in total immune cell number and cell-surface antigen expression in the thymus, spleen and bone marrow of B6C3F1 mice. *Vet Hum Toxicol* 37: 99-104.

Hogstedt, C; Jansson, C; Hugosson, M; Tinnerberg, H; Gustavsson, P. (2013). Cancer incidence in a cohort of Swedish chimney sweeps, 1958-2006. *Am J Public Health* 103: 1708-1714.

Howlader N, Noone AM, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z,Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2012, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2012/, based on November 2014 SEER data submission, posted to the SEER web site, April 2015.

Hsu, PC; Chen, IY; Pan, CH; Wu, KY; Pan, MH; Chen, JR; Chen, CJ; Chang-Chien, GP; Hsu, CH; Liu, CS; Wu, MT. (2006). Sperm DNA damage correlates with polycyclic aromatic hydrocarbons biomarker in coke-oven workers. *Int Arch Occup Environ Health* 79: 349-356.

Hussain, SP; Amstad, P; Raja, K; Sawyer, M; Hofseth, L; Shields, PG; Hewer, A; Phillips, DH; Ryberg, D; Haugen, A; Harris, CC. (2001) Mutability of p53 hotspot codons to bnezo[a]pyrene diol epoxide (BPDE) and the frequency of p53 mutations in nontumorous human lung. *Cancer Research* 61:6350-6355.

IARC (International Agency for Research on Cancer). (2003). Predictive value of rodent forestomach and gastric neuroendocrine tumours in evaluating carcinogenic risks to humans: Views and expert opinions of an IARC working group, Lyon, 29 November – 1 December 1999. (IARC Technical Publication No. 39), Lyon, France.

IARC (International Agency for Research on Cancer). (2010). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monograph, Vol. 100F, pp.1-853. Lyon, France. http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-21.pdf

J AM; Jennette, KW; Blobstein, SH; Weinstein, IB; Beland, FA; Harvey, RG; Kasai, H; Miura, K; Nakanishi, K. (1976) Benzo[a]pyrene-nucleic acid derivative found in vivo: structure of a benzo[a]pyrene-tetrahydrodiol epoxide-guanine adduct. *Journal of the American Chem. Soc.* 98:5714-5.

Jayasundara, N; Van Tiem Garner, L; Meyer, JN; Erwin, KN; and Di Giulio, RT. (2015). AHR2-Mediated Transcriptomic Responses Underlying the Synergistic Cardiac Developmental Toxicity of PAHs. *Tox Sci* 143(2):469-81.

Jemec, GBE; Osterlind, A. (1994). Cancer in patients treated with coal tar: a long-term follow up study. *J of the European Academy of Dermatology & Venerology* 3: 153-156.

Jeng HA, Pan CH, Diawara N , Chang-Chien GP , Lin WY , Huang CT , et al . (2011). Polycyclic aromatic hydrocarbon – induced oxidative stress and lipid peroxidation in relation to immunological alteration. *Occup Environ Med* 68: 653 – 8.

Jeng, H.A., D. Yordt, S. Davis, and J.R. Swanson. 2015. Assessment of alteration of reproductive system in vivo induced by subchronic exposure to benzo(a)pyrene via oral administration. *Environmental Toxicology*. 30:1-8.

Jones, SK; Mackie, RM; Hole, DJ; Gillis, CR. (1985). Further evidence of the safety of tar in the management of psoriasis. *British Journal of Dermatology* 113: 97-101.

Karakaya, A, Ates, I, Yucesoy, B. (2004). Effects of occupational polycyclic aromatic hydrocarbon 3 exposure on T-lymphocyte functions and natural killer cell activity in asphalt and coke oven 4 workers. Hum Exp Toxicol 23: 317-322.

Karakaya, A; Yücesoy, B; Turhan, A; Erdem, O; Burgaz, S; Karakaya, AE. (1999). Investigation of some immunological functions in a group of asphalt workers exposed to polycyclic aromatic hydrocarbons. *Toxicology* 135: 43-47.

Karlehagen, S; Andersen, A; Ohlson, CG. (1992). Cancer incidence among cresote-exposed workers. *Scand J Work Environ Health* 18: 26-29.

King, HWS; Osborne, MR; Beland, FA; Harvey, RG; and Brookes, P. (1976) 7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene is an intermediate in the metabolism and binding to DNA of benzo[a]pyrene, *Proc Natl Acad Sci USA* 73:2679-2681.

Knaapen, AM; Curfs, DM; Pachen, DM; Gottschalk, RW; de Winther MP; Daemen MJ; Van Schooten FJ . (2007). The environmental carcinogen benzo[*a*]pyrene induces expression of monocyte-chemoattractant protein-1 in vascular tissue: a possible role in atherogenesis . *Mutat Res* 621: 31 – 41.

Knuckles, ME; Inyang, F; Ramesh, A. (2001). Acute and subchronic oral toxicities of benzo[a]pyrene in F-344 rats. *Toxicol Sci* 61: 382-388.

Kong, LY, Luster, MI, Dixon, D, O'Grady, J, Rosenthal, GJ. (1994). Inhibition of lung immunity after intratracheal instillation of benzo(a)pyrene. *Am J Respir Crit Care Med* 150: 1123-1129.

Kroese, ED; Muller, JJA; Mohn, GR; Dortant, PM; Wester, PW. (2001). Tumorigenic effects in Wistar rats orally administered benzo[a]pyrene for two years (gavage studies): Implications for human cancer risks associated with oral exposure to polycyclic aromatic hydrocarbons. (658603 010). Bilthoven, The Netherlands: National Institute for Public Health and the Environment (RIVM). http://www.rivm.nl/bibliotheek/rapporten/658603010.pdf

Kyrtopoulos, SA. (2006). Biomarkers in environmental carcinogenesis research: striving for a new momentum. *Tox Lett* 162:3-15.

Kummer, V., J. Maskova, Z. Zraly, and M. Faldyna. 2013. Ovarian disorders in immature rats after postnatal exposure to environmental polycyclic aromatic hydrocarbons. *Journal of Applied Toxicology*. 33:90-99.

Ladics, GS, Kawabata, TT, Munson, AE, White, KL Jr. (1992). Evaluation of murine splenic cell type metabolism of benzo[a]pyrene and functionality in vitro following repeated in vivo exposure to benzo[a]pyrene. *Toxicol Appl Pharmacol*. 16:258-266.

Levin, W; Wood, AW; Wislocki, PG; Kapitulnik, J; Yagi, H; Jerina, DM; Conney, Ah. (1977). Carcinogenicity of benzo-ring derivatives of benzo(a)pyrene on mouse skin. *Cancer Res* 37: 3356-3361.

Li Z, Chadalapaka G, Ramesh A, Khoshbouei H, Maguire M, Safe S, Rhoades RE, Clark R, Jules G, McCallister M, Aschner M, Hood DB (2012) PAH particles perturb prenatal processes and phenotypes: protection from deficits in object discrimination afforded by dampening of brain oxidoreductase following in utero exposure to inhaled benzo(a)pyrene. *Toxicol Sci* 125:233-247.

Liang, J.R., H.Y. Zhu, C.Z. Li, Y.C. Ding, Z.J. Zhou, and Q. Wu. 2012. Neonatal exposure to benzo a pyrene decreases the levels of serum testosterone and histone H3K14 acetylation of the StAR promoter in the testes of SD rats. *Toxicology*. 302:285-291.

Liu, SH; Wang, JH; Chuu, JJ; Lin-Shiau, SY. (2002). Alterations of motor nerve functions in animals exposed to motorcycle exhaust. *J Toxicol Environ Health A* 65: 803-812.

Lu LJ, Disher RM, Reddy MV, Randerath K. (1986) 32P-postlabeling assay in mice of transplacental DNA damage induced by the environmental carcinogens safrole, 4-aminobiphenyl, and benzo(a)pyrene. Cancer Res.46(6):3046-54.

Luebke, RW; Chen, DH; Dietert, R; Yang, Y; King, M; Luster, MI. (2006). The comparative immunotoxicity of five selected compounds following developmental or adult exposure [Review]. *J Toxicol Environ Health B Crit Rev* 9: 1-26. http://dx.doi.org/10.1080/15287390500194326

Luster MI, Portier C, Pait DG, White KL Jr, Gennings C, Munson AE, Rosenthal GJ. (1992) Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. *Fundam Appl Toxicol* 18:200-210.

Maciel ES, Biasibetti R, Costa AP, Lunardi P, Schunck RV, Becker GC, Arbo MD, Dallegrave E, Goncalves CA, Saldiva PH, Garcia SC, Leal RB, Leal MB (2014) Subchronic oral administration of Benzo[a]pyrene impairs motor and cognitive behavior and modulates S100B levels and MAPKs in rats. *Neurochem Res* 39:731-740.

Mackenzie, KM; Angevine, DM. (1981). Infertility in mice exposed in utero to benzo(a)pyrene. Biol Reprod 24: 183-192. Manchester, DK; Weston, A; Choi, J-S; Trivers, GE; Fennessey, PV; Quintana, E; Farmer, PB; Mann, DL; and Harris, CC. (1988) Detection of benzo[a]pyrene diol-epoxide-DNA adducts in human placenta. *Proc. Natl. Acad. Sci. USA.*, 85: 9243-9247.

Markham JA, Taylor AR, Taylor SB, Bell DB, Koenig JI. 2010. Characterization of the cognitive impairments induced by prenatal exposure to stress in the rat. *Frontiers in Behavioral Neuroscience* 4:1–15.

Marshall, CJ; Vousden KH; Phillips, DH. (1984) Activation of c-Ha-*ras*-1 proto-oncogene by *in vitro* modification with a chemical carcinogen, benzo[a]pyrene diol-epoxide. *Nature* 310:586-589.

Mattison, D.R. 1980. Morphology of oocyte and follicle destruction by polycyclic aromatic hydrocarbons in mice. *Toxicology and Applied Pharmacology*. 53:249-259.

Mattison, D.R., N.B. White, and M.R. Nightingale. 1980. The effect of benzo(a)pyrene on fertility, primordial oocyte number, and ovarian response to pregnant mare's serum gonadotropin. *Pediatr Pharmacol (New York)*. 1:143-151.

McCallister MM, Maguire M, Ramesh A, Aimin Q, Liu S, Khoshbouei H, Aschner M, Ebner FF, Hood DB (2008) Prenatal exposure to benzo(a)pyrene impairs later-life cortical neuronal function. *Neurotoxicology* 29:846-854.

Menter, A; Cram, DL. (1983). The Goeckerman regimen in two psoriasis day care centers. J Am Acad Dermatol 9: 59-65.

Miller, BG; Doust, E; Cherrie, JW; Hurley, JF. (2013) Lung cancer mortality and exposure to polycyclic aromatic hydrocarbons in British coke oven workers. *BMC Public Health* 13:962.

Mohamed, E; Song, WH; Oh, SA; Park, YI; You, YA; Lee, S; Choi, JY; Kim, YJ; Jo, I; Pang, MG. (2010). The transgenerational impact of benzo(a)pyrene on murine male fertility. *Hum Reprod 25*: 2427-2433.

Moorthy, B; Chu C; Carlin, DJ. (2015). Contemporary Review. Polycyclic Aromatic Hydrocarbons: From Metabolism to Lung Cancer. *Tox Sci* 145(1):5-15.

Muller, SA; Kierland, RR. (1964). Crude coal tar in dermatologic therapy. *Mayo Clinic Proceedings* 39: 275-280.

Munson, AE; White, KL; Lysy, HH. (1985). Effects of Subchronic Fourteen Day Exposure to Benzopyrene 23 in B6C3F1 Female Mice on Host Resistance. Submitted under TSCA Section FYI.

Nakamura, BN; Mohar, I; Lawson, GW; Cortes, MM; Hoang, YD; Ortiz, L; Patel, R; Rau, BA; McConnachie, LA; Kavanagh, TJ; and Luderer, U. (2012). Increased sensitivity to testicular

toxicity of transplacental benzo[a]pyrene exposure in male glutamate cysteine ligase modifier subunit knockout (Gc;,-/-) mice. *Toxicological Sciences* 126:227-241.

Nanez, A., Alejandro, N.F., Falahatpisheh, M.H., Roths, J.B. and Ramos, K.S. (2005). Disruption of cell-cell and cell-matrix interactions in hydrocarbon nephropathy. *American Journal of Physiology-Renal* 289(6), F1291-F1303. Epub 2005 Jul 5. PMID: 15998846.

Nanez, A., Ramos, I.N. and Ramos, KS (2011). A mutant allele of AHR protects the embryonic kidney from hydrocarbon-induced deficits in fetal programming. *Environmental Health Perspectives* 119, 1745-1753. PMID 21803694.

Nebert DW, Levitt RC, Jensen NM, Lambert GH, Felton JS. 1977 Birth defects and aplastic anemia: differences in polycyclic hydrocarbon toxicity associated with the Ah locus. *Arch Toxicol*. Dec 30;39(1-2):109-32.

Nesnow, S; Triplett, LL; Slaga, TJ. (1983). Mouse skin tumor initiation-promotion and complete carcinogenesis bioassays: mechanisms and biological activities of emission samples. *Environ Health Perspect* 47: 255-268.

N'Diaye, M; Le Ferrec, E; Lagadic-Gossmann, D; Corree, S; Gilot, D; Lecureur, V; Monteiro, P; Rauch, C; Galibert, MD; Fardel, O. (2006). Aryl hydrocarbon receptor- and calcium-dependent induction of the chemokine CCL1 by the environmental contaminant benzo[a]pyrene. *J. Biol Chem* 281: 19906-19915.

N ' Diaye, M; Le Ferrec, E; Kronenberg, F; Dieplinger, H; Le Vee, M; Fardel, O. (2009). TNF α - and NF- κ B-dependent induction of the chemokine CCL1 in human macrophages exposed to the atherogenic lipoprotein(a). *Life Sci* 84:451 – 7.

Niu, Q; Zhang, H; Li, X; Li, M. (2010). Benzo[a]pyrene-induced neurobehavioral function and neurotransmitter alterations in coke oven workers. *Occup Environ Med* 67: 444-448.

NRC (National Research Council) (2014). Review of EPA's Integrated Risk Information System (IRIS Process). National Research Council, Washington, DC.

Olsen, A.-K., Å. Andreassen, R. Singh, R. Wiger, N. Duale, P.B. Farmer, and G. Brunborg. 2010. Environmental exposure of the mouse germ line: DNA adducts in spermatozoa and formation of <italic>de novo</italic> mutations during spermatogenesis. *PLoS ONE*. 5:e11349.

Osborne, MR; Thompson, MH; Tarmy, EM; Beland, FA; Harvey, RG; Brookes, P. (1976) The reaction of 7,8-dihydro-7,8-dihydroxybenzo[a]pyrene-9,10-oxide with DNA in relation to the benzo[a]pyrene-DNA products isolated from cells. *Chem.-Biol. Interactions* 13:343-348.

Oesterling E, Toborek M, Hennig B . (2008). Benzo[*a*]pyrene induces intercellular adhesion molecule-1 through a caveolae and aryl hydrocarbon receptor mediated pathway. *Toxicol Appl Pharmacol*, 232 , 309 – 16.

Parrish, A.R., Alejandro, N.F., Bral, C.M., Kerzee, J.K., Bowes, R.C.III and Ramos, K.S. Characterization of glomerular cell phenotypes following repeated cycles of BaP injury in vitro. *Biochemical Pharmacology* 64(1), 31-39, Jul 2002. PMID: 12106603

Partanen, T; Boffetta, P. (1994). Cancer risk in asphalt workers and roofers: review and meta-analysis of epidemiologic studies [Review*]*. *Am J Ind Med* 26: 721-740.

Patri M, Singh A, Mallick BN (2013) Protective role of noradrenaline in benzo[a]pyrene-induced learning impairment in developing rat. *J Neurosci Res* 91:1450-1462.

Pauluhn, J; Thyssen, J; Althoff, J; Kimmerle, G; Mohr, U. (1985). Long-term inhalation study with benzo(a)pyrene and SO2 in Syrian golden hamsters. *Exp. Path.* 28:31.

Perera F, Li TY, Lin C, Tang D (2012a) Effects of prenatal polycyclic aromatic hydrocarbon exposure and environmental tobacco smoke on child IQ in a Chinese cohort. *Environ Res* 114:40-46.

Perera FP, Chang HW, Tang D, Roen EL, Herbstman J, Margolis A, Huang TJ, Miller RL, Wang S, Rauh V (2014) Early-life exposure to polycyclic aromatic hydrocarbons and ADHD behavior problems. PLoS ONE 9:e111670.

Perera FP, Li Z, Whyatt R, Hoepner L, Wang S, Camann D, Rauh V (2009) Prenatal airborne polycyclic aromatic hydrocarbon exposure and child IQ at age 5 years. *Pediatrics* 124:e195-e202.

Perera FP, Rauh V, Whyatt RM, Tang D, Tsai WY, Bernert JT, Tu YH, Andrews H, Barr DB, Camann DE, Diaz D, Dietrich J, Reyes A, Kinney PL (2005) A summary of recent findings on birth outcomes and developmental effects of prenatal ETS, PAH, and pesticide exposures. *Neurotoxicology* 26:573-587.

Perera FP, Rauh V, Whyatt RM, Tsai WY, Bernert JT, Tu YH, Andrews H, Ramirez J, Qu L, Tang D (2004) Molecular evidence of an interaction between prenatal environmental exposures and birth outcomes in a multiethnic population. *Environ Health Perspect* 112:626-630.

Perera FP, Tang D, Wang S, Vishnevetsky J, Zhang B, Diaz D, Camann D, Rauh V (2012b) Prenatal polycyclic aromatic hydrocarbon (PAH) exposure and child behavior at age 6-7 years. *Environ Health Perspect* 120:921-926.

Perera FP, Wang S, Vishnevetsky J, Zhang B, Cole KJ, Tang D, Rauh V, Phillips DH (2011) Polycyclic aromatic hydrocarbons-aromatic DNA adducts in cord blood and behavior scores in New York city children. *Environ Health Perspect* 119:1176-1181.

Poirier, MC. (2012). Chemical-induced DNA damage and human cancer risk. *Discovery Medicine* 14(77):283-288.

Pratt, MM; John, K; MacLean, AB; Afework, S; Phillips, DH; and Poirier, MC. (2011). Polycyclic aromatic hydrocarbon (PAH) exposure and DNA adduct semi-quantitation in archived human tissues. *International J Environmental Research and Public Health* 8:2675-2691.

Qiu, C; Cheng, S; Xia, Y; Peng, B; Tang, Q; Tu, B. (2011). Effects of subchronic benzo(a)pyrene exposure on neurotransmitter receptor gene expression in the rat hippocampus related with spatial learning and memory change. *Toxicology* 289: 83-90.

Qiu, C: Peng, B; Cheng, S; Xia, Y; Tu, B. (2013). The effect of occupational exposure to benzo[a]pyrene on neurobehavioral function in coke oven workers. *Am J Ind Med* 56: 347-355.

Raiz J, Damert A, Chira S, Held U, Klawitter S, Hamdorf M, Löwer J, Strätling WH , Löwer R, Schumann GG. (2012). The non-autonomous retrotransposon SVA is trans-mobilized by the human LINE-1 protein machinery. *Nucleic Acids Research* 40(4): 1666-1683.

Ramesh, A; Invang, F; Lunstra, DD; Niaz, MS; Kopsombut, P; Jones, KM; Hood, DB; Hills, ER; Archibong, AE. (2008). Alteration of fertility endpoints in adult male F-344 rats by subchronic exposure to inhaled benzo(a)pyrene. *Exp Toxicol Path* 60: 269-280.

Ramos, K.S., Teneng, I., Montoya-Durango, D.E., Bojang, P., Haeberle, M.T., Ramos, I.N., Stribinskis, V. and Kalbfleisch, T. (2013). The Intersection of Genetics and Epigenetics: Reactivation of Mammalian LINE-1 Retrotransposons by Environmental Injury. In *Environmental Epigenomics in Health and Disease: Epigenetics and Disease Origins.* Vol. 1, Heidelberg: Springer, 2013.

Rigdon RH, Rennels EG. (1964) Effect of feeding benzpyrene on reproduction in the rat. *Experientia*. Apr 15;20(4):224-6.

Rodriguez, JW; Kirlin, WG; Wirsly, YG; Matheravidathu, S; Hodge, TW; Urso, P. (1999). Maternal exposure to benzo[a]pyrene alters development of T lymphocytes in offspring. *Immunopharmacol Immunotoxicol* 21: 379-396.

Romundstad, P; Andersen, A; Haldorsen, T. (2000). Cancer incidence among workers in six Norwegian aluminum plants. *Scand J Work Environ Health* 26:461-469.

Ronneberg, A; Haldorsen, T; Romundstad, P; Andersen, A. (1999). Occupational exposure and cancer incidence among workers from an aluminum smelter in western Norway. *Scand J Work Environ Health* 25:207-214.

Rota, M; Bosetti, C; Boccia, S; Boffetta, P; La Vecchia, C. (2014). Occupational exposures to polycyclic aromatic hydrocarbons and respiratory and urinary tract cancers: an updated systematic review and a meta-analysis to 2014. *Arch Toxicol* 88(8):1479-90.

Rothman, N; Correa-Villasenor, A; Ford, DP; Poirier, MC; Haas, R; Hansen, JA; O'Toole, T; Strickland, PT. (1993). Contribution of occupation and diet to white blood cell polycyclic aromatic hydrocarbon-DNA adducts in wildland firefighters. *Cancer Epidemiology Biomarkers and Prevention* 2:341-347.

Roy, TA and Singh, R. (2011). Effect of soil loading and soil sequestration on dermal bioavailability of polynuclear aromatic hydrocarbons. *Bull Environ Contam Toxicol*. 67(3):324-331.

Sadeu, J.C., and W.G. Foster. 2011. Effect of in vitro exposure to benzo a pyrene, a component of cigarette smoke, on folliculogenesis, steroidogenesis and oocyte nuclear maturation. *Reproductive Toxicology*. 31:402-408.

Sadeu, J.C., and W.G. Foster. 2013. The cigarette smoke constituent benzo a pyrene disrupts metabolic enzyme, and apoptosis pathway member gene expression in ovarian follicles. *Reproductive Toxicology*. 40:52-59.

Saunders, CR; Shockley, DC; Knuckles, ME. (2001). Behavioral effects induced by acute exposure to benzo(a)pyrene in F-344 rats. *Neurotox Res* 3: 557-579.

Saunders, CR; Ramesh, A; Shocklye, DC. (2002).Modulation of neurotoxic behavior in F-344 rats by temporal disposition of benzo(a)pyrene. *Toxicol Lett* 129: 33-45.

Saunders, CR; Das, SK; Ramesh, A; Shocklyey, Dc; Mukherjee, S. (2006). Benzo(a)pyrene-induced acute neurotoxicity in the F-344 rat: role of oxidative stress. *J Appl Toxicol* 26: 427-438.

Schlede E, Merker HJ. (1972) Effect of benzo( )pyrene treatment on the benzo( )pyrene hydroxylase activity in maternal liver, placenta, and fetus of the rat during day 13 to day 18 of gestation. *Naunyn Schmiedebergs Arch Pharmacol* 272(1):89-100.

Shendrikova IA, Aleksandrov VA. (1974) Comparative penetration of polycyclic hydrocarbons through the rat placenta into the fetus. *Bull Exp Biol Med.* 77(2):169-71.

Sheng, L; Ding, X; Ferguson, M; McCallister, M; Rhoades, R; Maguire, M; Ramesh, A; Aschner, M; Campbell, D; Levitt, P; Hood, DF. (2010). Prenatal polycyclic aromatic hydrocarbon exposure leads to behavioral deficits and downregulation of receptor tyrosine kinase, MET. *Toxicol Sci* 118: 625-634.

Shum S, Jensen NM, Nebert DW. (1979) The murine Ah locus: in utero toxicity and teratogenesis associated with genetic differences in benzo[a]pyrene metabolism. *Teratology* 20(3):365-76.

Sims, P; Grover, PL; Swaisland, A; Pal, K; and Hewer, A. (1974). Metabolic activation of benzo[a]pyrene proceeds by a diol-epoxide, *Nature* 252:236-327.

Sinha, R; Kulldorff, M; Gunter, MJ; Strickland, P; Rothman, N. (2005). Dietary benzo[a]pyrene intake and risk of colorectal adenoma. *Cancer Epidemiology Biomarkers and Prevention* 14(8):2030-2034.

Sivak, A; Niemeier, R; Lynch, D; Beltis, K; Simon, S; Salomon, R; Latta, R. Belinky, B; Menzies, K; Lunsford, A; Cooper, C; Ross, A; Bruner, R. (1997). Skin carcinogenicity of condensed asphalt roofing fumes and their fractions following dermal application to mice. *Cancer Lett* 117: 113-123.

Soares, SR; Melo, MA.(2008). Cigarette smoking and reproductive function [Review]. *Curr Opin Obstet Gynec*ol 20: 281-291.

Spinelli, JJ; Band, PR; Svirchev, LM; Gallagher, RP. (1991). Mortality and cancer incidence in aluminum reduction plant workers. *J Occup Med* 33:1150-1155.

Spinelli, JJ; Demers, PA; Le, ND; Friesen, MD; Lorenzi, MF; Fang, R; Gallagher, RP (2006). Cancer risk in aluminum reduction plant workers (Canada). *Cancer Causes Control* 17: 939-948.

Starostenko, L.V., N.I. Rechkunova, N.A. Lebedeva, A. Kolbanovskiy, N.E. Geacintov, and O.I. Lavrik. 2014. Human DNA polymerases catalyze lesion bypass across benzo[a]pyrene-derived DNA adduct clustered with an abasic site. *DNA Repair*. 24:1-9.

Sticha, KRK; Staretz, ME; Wang, M; Liang, H; Kenney, PMJ; Hecht, SS. (2000) Effects of benzyl isothiocyanate and phenyl isothiocyanate on benzo[a]pyrene metabolism and DNA adduct formation in the A/J mouse. *Carcinogenesis* 21:1711-1719.

Stribinskis, V; Ramos, KS. (2006). Activation of human LINE-1 retrotransposition by benzo(a)pyrene. *Cancer Research* 66(5):2616-2620.

Swaen, GMH; Slangen, JJM; Volovics, A; et al. (1991). Mortality of coke plant workers in the Netherlands. *Br J Ind Med* 48:130-135.

Swartz, W.J., and D.R. Mattison. 1985. Benzo(a)pyrene inhibits ovulation in C57BL/6N mice. *Anat Rec*. 212:268-276.

Szczeklik, A, Szczeklik, J, Galuszka, Z, Musial, J, Kolarzyk, E, Targosz, D. (1994). Humoral immunosuppression in men exposed to polycyclic aromatic hydrocarbons and related carcinogens in polluted environments. *Environ Health Perspect* 102: 302-304.

Tang D, Li TY, Liu JJ, Chen YH, Qu L, Perera F (2006) PAH-DNA adducts in cord blood and fetal and child development in a Chinese cohort. *Environ Health Perspect* 114:1297-1300.

Tang D, Li TY, Liu JJ, Zhou ZJ, Yuan T, Chen YH, Rauh VA, Xie J, Perera F (2008) Effects of prenatal exposure to coal-burning pollutants on children's development in China. *Environ Health Perspect* 116:674-679.

Tang Q, Xia Y, Cheng S, Tu B (2011) Modulation of behavior and glutamate receptor mRNA expression in rats after sub-chronic administration of benzo(a)pyrene. *Biomed Environ Sci* 24:408-414.

Temple L, Kawabata TT, Munson AE, White KL Jr. (1993) Comparison of ELISA and plaque-forming cell assays for measuring the humoral immune response to SRBC in rats and mice treated with benzo[a]pyrene or cyclophosphamide. *Fundam Appl Toxicol* 21:412-419.

Teneng, I.; Montoya-Durango, D.E.; Quertermous, J.; Lacy, M.E.; Ramos, K.S. Reactivation of L1 retrotransposon by benzo(a)pyrene involves complex genetic and epigenetic regulation. *Epigenetics* 6, 355-367, 2011. PMID 21150308.

Thakur VS, Liang YW, Lingappan K, Jiang W, Wang L, Barrios R, Zhou G, Guntupalli B, Shivanna B, Maturu P, Welty SE, Moorthy B, Couroucli XI. (2014) Increased susceptibility to hyperoxic lung injury and alveolar simplification in newborn rats by prenatal administration of benzo[a]pyrene..*Toxicol Lett*. ;230(2):322-32.

Thyssen, J; Althoff, J; Kimmerle, G; Mohr, U. (1981). Inhalation studies with benzo(a)pyrene in Syrian golden hamsters. *J. Natl Cancer Inst* 66: 575-577.

Tornqvist, S; Norell, S; Ahlbom, A; Knave, B. (1986). Cancer in the electric power industry. *Br J Ind Med* 43: 212-213.

Uno et al. (2014). Protective role of cytochrome P450 1A1 (CYP1A1) against benzo[a]pyrene-induced toxicity in mouse aorta. *Toxicology* 316:34-42.

Urso, P, Gengozian, N. (1982). Alterations in the humoral immune response and tumor frequencies in mice exposed to benzo[a]pyrene and X-rays before or after birth. *J Toxicol Environ Health* 10:817-835. http://dx.doi.org/10.1080/15287398209530297

Urso, P, Gengozian, N. (1984). Subnormal expression of cell-mediated and humoral immune responses 22 in progeny disposed toward a high incidence of tumors after in utero exposure to benzo[a]pyrene. *J Toxicol Environ Health* 14: 569-584. http://dx.doi.org/10.1080/15287398409530606

Urso, P, Johnson, RA. (1987). Early changes in T lymphocytes and subsets of mouse progeny defective as adults in controlling growth of a syngeneic tumor after in utero insult with benzo(a)pyrene. *Immunopharmacology* 14: 1-10.

U.S. EPA (1990). Development of relative potency estimates for PAHs and hydrocarbon combustion product fractions compared to benzo[a]pyrene and their use in carcinogenic risk assessment. (EPA/600/R-92/134). Washington, DC. U.S. Environmental Protection Agency.

U.S.EPA (2005a). Guidelines for Carcinogen Risk Assessment. (EPA/630/P-03/0001F). Washington, DC U.S. Environmental Protection Agency, Risk Assessment Forum.

U.S.EPA (2005b). Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. (EPA/630/R-03/003F). Washington, DC. U.S. Environmental Protection Agency. Risk Assessment Forum.

U.S.EPA (2011). Recommended use of body weight$^{3/4}$ as the default method in derivation of the oral reference dose. (EPA/100/R11/0001). Washington, DC. U.S. Environmental Protection Agency, Risk Assessment Forum.

Valentovic, M.A., Alejandro, N, Brown, P.I. and Ramos, K.S. (2006). Streptozotocin (STZ) diabetes enhances BaP-induced renal injury in Sprague Dawley rats. *Toxicology Letters* 164(3), 214-220. Epub 2006 Feb 7. PMID: 16460892.

VanGijssel, HE; Schild, LJ; Watt, DL; Roth, MJ; Wang, GQ; Dawsey, SM; Albert, PS; Qiao, YL; Taylor, PR; Dong, Z-W; and Poirier, MC (2004). Polycyclic aromatic hydrocarbon-DNA adducts determined by semiquantitative immunohistochemistry in human esophageal biopsies taken in 1985. *Mut. Res.* 547:55-62.

Verhofstad, N., J. La Pennings, C.T.M. van Oostrom, J. van Benthem, F.J. van Schooten, H. van Steeg, and R.W.L. Godschalk. 2010a. Benzo(a)pyrene induces similar gene expression changes in testis of DNA repair proficient and deficient mice. *Bmc Genomics*. 11.

Verhofstad, N., C.T.M. van Oostrom, J. van Benthem, F.J. van Schooten, H. van Steeg, and R.W.L. Godschalk. 2010c. DNA Adduct Kinetics in Reproductive Tissues of DNA Repair Proficient and Deficient Male Mice After Oral Exposure to Benzo(a)pyrene. *Environmental and Molecular Mutagenesis*. 51:123-129.

Verhofstad, N., C.T.M. van Oostrom, E. Zwart, L.M. Maas, J. van Benthem, F.J. van Schooten, H. van Steeg, and R.W.L. Godschalk. 2011. Evaluation of Benzo(a)pyrene-Induced Gene Mutations in Male Germ Cells. *Toxicological Sciences*. 119:218-223.

Wei, D; Maher, VM; McCormick, JJ. (1995). Site-specific rates of excision repair of benzo[a]pyrene diol epoxide adducts in the hypoxanthine phosphoribosyltransferase gene in human fibroblasts: Correlation with mutation spectra. *Proc. Natl. Acad. Sci. USA* 92:2204-2208.

Wells PG, Kim PM, Laposa RR, Nicol CJ, Parman T, Winn LM. (1997) Oxidative damage in chemical teratogenesis. *Mutat Res.* 396(1-2):65-78.

Wester P, Muller J , Slob W , Mohn G , Dortant P , Kroese E . (2012). Carcinogenic activity of benzo[*a*]pyrene in a 2 year oral study in Wistar rats. *Food Chem Toxicol*, 50, 927– 35.

WHO (World Health Organization). (2012). Guidance for immunotoxicity risk assessment for chemicals. (Harmonization Project Document No. 10). Geneva, Switzerland. http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf

Winker, N; Tuschl, H; Kovac, R; Weber, E. (1997). Immunological investigations in a group of workers exposed to various levels of polycyclic aromatic hydrocarbons. J Appl Toxicol 17: 23-29.

Wormley, DD; Chirwa, S; Navvar, T; Wu, J; Johnson, S; Brown, LA; Harris, E; Hood, DC. (2004). Inhaled benzo[a]pyrene impairs long-term potentiation in the F1 generation rat dentate gyrus. *Cell Mol Biol* (Noisy-le-grand) 50: 715-721.

Wu, J; Ramesh, A; Nayyar, T; Hood, DB. (2003). Assessment of metabolites and AhR and CYP1A1 mRNA expression subsequent to prenatal exposure to inhaled benzo(a)pyrene. *Int. J. Dev. Neurosci.* 21:333-346.

Wu, J; Hou, H; Ritz, B; Chen, Y. (2010). Exposure to polycyclic aromatic hydrocarbons and missed abortion in early pregnancy in a Chinese population. *Sci Total Environ* 408: 2312-2318.

Xia, Y; Cheng, S; He, J; Liu, X; Tang, Y; Yuan, H; He, L; Lu, T; Tu, B; Wang, Y. (2001). Effects of subchronic exposure to benzo[a]pyrene (B[a]P on learning and memory, and neurotransmitters in male Sprague-Dawley rat. *Neurotoxicology* 32: 188-198.

Xu, Z; Brown, LM; Pan, GW; et al. (1996). Cancer risks among iron and steel workers in Anshan, China, Part II: Case-control studies of lung and stomach cancer. *Am J Ind Med* 30:7-15.

Xu, C., Chen, J.-A., Qiu, Z., Zhao, Q., Luo, J., Yang, L., Zeng, H., Huang, Y., Zhang, L., Cao, J., and Shu, W. (2010). Ovotoxicity and PPAR-mediated aromatase downregulation in female Sprague-Dawley rats following combined oral exposure to benzo[a]pyrene and di-(2-ethylhexyl)phthalate. *Toxicology Letters*. 199:323-332.

Xu, G., C.A. McMahan, and C.A. Walter. (2014). Early-Life exposure to benzo[a]pyrene increases mutant frequency in spermatogenic cells in adulthood. *PLoS ONE*. 9:e87439.

Yang H , Zhou L , Wang Z , Roberts LJ II , Lin X , Zhao Y , Guo Z . (2009). Overexpression of antioxidant enzymes in ApoE-deficient mice suppresses benzo(*a*)pyrene-accelerated atherosclerosis. *Atherosclerosis* 207, 51 – 8.

Young, R., H. Dinesdurage, M. McKeon, D. Bruning, M. Aardema, and R. Kulkarni. 2014. Qualification and comparison of Big Blue® transgenic mouse and rat mutation assays with n-ethyl-n-nitrosourea (ENU) and benzo(a)pyrene (BaP). *Toxicology Letters*. 229, Supplement:S56.

Zanardi, F; Salvarani, R; Cooke, RM; Pirastu, R; Baccini, M; Christiani, D; Curti, S; Risi, A; Barbieri, A; Barbieri, G; Mattioli, S; Violante, FS. (2013) Carcinoma of the pharynx and tonsils in an occupational cohort of asphalt workers. *Epidemiology* 24:100–103.

Zhang, JM; Nie, JS; Li, X; Niu, O. (2012). Characteristic analysis of peripheral blood mononuclear cell apoptosis in coke oven workers. *J. Occup Health* 54: 44-50.

APPENDIX A: EPA'S CHARGE QUESTIONS

Charge to the Science Advisory Board for the IRIS Toxicological Review of Benzo[a]pyrene

September 2014 (Updated March 2015[1])

Introduction

The U.S. Environmental Protection Agency (EPA) is seeking a scientific peer review of a draft Toxicological Review of Benzo[a]pyrene developed in support of the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD).

IRIS is a human health assessment program that evaluates scientific information on effects that may result from exposure to specific chemical substances in the environment. Through IRIS, EPA provides high quality science-based human health assessments to support the Agency's regulatory activities and decisions to protect public health. IRIS assessments contain information for chemical substances that can be used to support hazard identification and dose- response assessment, two of the four steps in the human health risk assessment process. When supported by available data, IRIS provides health effects information and toxicity values for health effects (including cancer and effects other than cancer) resulting from chronic exposure. IRIS toxicity values may be combined with exposure information to characterize public health risks of chemical substances; this risk characterization information can then be used to support risk management decisions.

An existing assessment for benzo[a]pyrene, which includes an oral slope factor (OSF) and a cancer weight of evidence descriptor, was posted on IRIS in 1987. The IRIS Program is conducting a reassessment of benzo[a]pyrene. The draft Toxicological Review of Benzo[a]pyrene is based on a comprehensive review of the available scientific literature on the noncancer and cancer health effects in humans and experimental animals exposed to benzo[a]pyrene. Additionally, appendices for chemical and physical properties, toxicokinetic information, summaries of toxicity studies, and other supporting materials are provided as *Supplemental Information* (see Appendices A to E) to the draft Toxicological Review.

The draft assessment was developed according to guidelines and technical reports published by EPA (see *Preamble*), and contains both qualitative and quantitative characterizations of the human health hazards for benzo[a]pyrene, including a cancer descriptor of the chemical's human carcinogenic

[1] The charge questions were modified (as shown in bold font) as a result of panel discussions during the March 4, 2015 preliminary teleconference

potential, noncancer toxicity values for chronic oral (reference dose, RfD) and inhalation (reference concentration, RfC) exposure, and cancer risk estimates for oral, inhalation, and dermal exposure.

Charge questions on the draft Toxicological Review

1. **Literature search/study selection and Evaluation.**

The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the *Literature Search Strategy/Study Selection and Evaluation* section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene

2. **Hazard identification**. In section 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with benzo[a]pyrene exposure. The draft assessment uses EPA's guidance documents (see http://www.epa.gov/iris/backgrd.html/) to reach the following conclusions.

2a. **Developmental toxicity** (sections 1.1.1, 1.2.1). The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human, animal and **mechanistic** studies support this conclusion?

2b. **Reproductive toxicity** (sections 1.1.2, 1.2.1). The draft assessment concludes that male and female reproductive effects are a human hazard of benzo[a]pyrene exposure. Do the available human,animal and **mechanistic** studies support this conclusion?

2c. **Immunotoxicity** (sections 1.1.3, 1.2.1). The draft assessment concludes that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Do the available human, animal and **mechanistic** studies support this conclusion?

2d. **Cancer** (sections 1.1.5, 1.2.2). The draft assessment concludes that benzo[a]pyrene is "carcinogenic to humans" by all routes of exposure. Do the available human, animal, and mechanistic studies support this conclusion?

2e. **Other types of toxicity** (section 1.1.4). The draft assessment concludes that the evidence does not support other types of noncancer toxicity as a potential human hazard. Are there other types of noncancer toxicity that can be credibly associated with benzo[a]pyrene exposure?

3. **Dose-response analysis**. In section 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with benzo[a]pyrene exposure in section 1, then proposes an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see http://www.epa.gov/iris/backgrd.html/) in the following analyses.

3a. **Oral reference dose for effects other than cancer** (section 2.1). The draft assessment proposes an overall reference dose of $3x10^{-4}$ mg/kg-d based on developmental toxicity during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific considerations that are **inherent** for exposures during a critical window of development?

3b. **Inhalation reference concentration for effects other than cancer** (section 2.2). The draft assessment proposes an overall reference concentration of $2x10^{-6}$ mg/m^3 based on decreased fetal survival during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.2.5) reflect the scientific considerations that are **inherent** for exposures during a critical window of development?

3c. **Oral slope factor for cancer** (section 2.3). The draft assessment proposes an oral slope factor of 1 per mg/kg-d based on alimentary tract tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

3d. **Inhalation unit risk for cancer** (section 2.4). The draft assessment proposes an inhalation unit risk of **0.6** per mg/m^3 based on a combination of several types of benign and malignant tumors in hamsters. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

3e. **Dermal slope factor for cancer** (section 2.5). The draft assessment proposes a dermal slope factor of 0.006 per ug/day based on skin tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and scaling from mice to humans? Does the method for cross-species scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?

3f. **Age-dependent adjustment factors for cancer** (section 2.6). The draft assessment proposes the application of age-dependent adjustment factors based on a determination that benzo[a]pyrene induces cancer through a mutagenic mode of action (see the mode-of-action analysis in section 1.1.5). Do the available mechanistic studies in humans and animals support a mutagenic mode of action for cancer induced by benzo[a]pyrene?

4. **Executive summary.** Does the executive summary clearly and appropriately present the major conclusions of the assessment?

5. **Charge question on the public comments**

In August 2013, EPA asked for public comments on an earlier draft of this assessment. Appendix G summarizes the public comments and this assessment's responses to them. Please comment on EPA's

responses to the scientific issues raised in the public comments. Please consider in your review whether there are scientific issues that were raised by the public as described in Appendix G that may not have been adequately addressed by EPA.

APPENDIX B: ADDITIONAL PEER-REVIEWED STUDIES ON HEALTH EFFECTS OF BaP

The SAB recommends the following additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene:

Abdel-Rahman, MS; Skowronski, GA; Turkall, RM. (2002). Assessment of the Dermal Bioavailability of Soil-Aged Benzo(a)Pyrene. *Hum Ecol Risk Assess* 8, 429-441.

Aboutabl, ME; Zordoky, BN; El-Kadi, AO. (2009). 3-Methylcholanthrene and benzo(a)pyrene modulate cardiac cytochrome P450 gene expression and arachidonic acid metabolism in male Sprague Dawley rats . *Br J Pharmacol* 158:1808 – 19.

Aboutabl, ME; Zordoky, BN; Hammock, BD; El-Kadi, AO. (2011). Inhibition of soluble epoxide hydrolase confers cardioprotection and prevents cardiac cytochrome P450 induction by benzo(a)pyrene. *J Cardiovasc Pharmacol* 57: 273– 81.

Alejandro, NF; Parrish, AR; Bowes III, RC; Burghardt, RC; Ramos, KS. (2000). Phenotypic profiles of cultural glomerular cells following repeated cycles of hydrocarbon injury. *Kidney International* 57(4): 1571-1580.

Alexandrov, K; Rojas, M; Geneste, O; Castegnaro, M; Camus, A; Petruzzelli, S; Gluntini, C; and Bartsch, H. (1992) An improved fluorometric assay for dosimetry of benzo[a]pyrene diol-epoxide-DNA adducts in smokers'lung: comparison with total bulky adducts and aryl hydrocarbon hydroxylase activity. *Cancer Research* 52: 6248-6253.

Archibong, AE; Ramesh, A; Inyang, F; Niaz, MS; Hood, DB; Kopsombut, P. (2012). Endocrine disruptive actions of inhaled benzo(a)pyrene on ovarian function and fetal survival in Fisher F-344 adult rats. *Reproductive Tox* 34:635-43.

Armstrong, BG; Gibbs, G. (2009) Exposure-response relationship between lung cancer and polycyclic aromatic hydrocarbons (PAHs). *Occup Environ Med* 66:740–746.

Armstrong, B; Hutchinson, E; Unwin, J; Fletcher, T. (2004). Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: a review and meta-analysis. *Environ Health Perspect* 112(9):970-8.

Behrens, T; Schill, W; Ahrens, W. (2009). Elevated cancer mortality in a german cohort of bitumen workers: extended follow-up through 2004. *J Occup Environ Hyg* 6:555–561.

Boffetta, P; Jourenkova, N; Gustavsson, P. (1997). Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. *Cancer Causes Control* 8:444-472.

Booth, ED; Loose, RW; Watson, WP. (1999). Effects of Solvent on DNA Adduct Formation in Skin and Lung of Cd1 Mice Exposed Cutaneously to Benzo(a)Pyrene. *Arch Toxicol* 73:316-322.

Bosetti, C; Boffetta, P; La Vecchia, C. (2007). Occupational exposures to polycyclic aromatic hydrocarbons, and respiratory and urinary tract cancers: a quantitative review to 2005. *Ann Oncol* 18(3):431-46.

Bostrom, CE; Gerde, P; Hanberg, A; Jernstrom, B; Johansson, C; Kyrklund, T; Rannug, A; Tornqvist, M; Victorin, K; Westerholm, R. (2002). Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ Health Perspect* 110:451-488.

Boysen, G; Hecht, SS. (2003) Analysis of DNA and protein adducts of benzo[a]pyrene in human tissues using structure-specific methods. *Mutation Res* 543:17-30.

Burchiel, SW; Burdick, AD; Melendez, KF; Lauer, FT; Davis, JW. (2005). Role Of Oxidant Stress In The Activation Of Growth Factor Signaling Pathways In Human Breast Epithelial Cells By Environmental Polycyclic Aromatic Hydrocarbons (PAHS). *Toxicol Sci* 84(1-S):62.

Burdick, AD; Davis, JD; Liu, KJ; Hudson, LG; Shi, H; Monske, ML; Burchiel, SW. (2003) Benzo[a]pyrene quinones increase cell proliferation, generate reactive oxygen species, and transactivate the epidermal growth factor receptor. *Cancer Research* 63:7825-7833.

Chen, S-Y; Wang, L-Y; Lunn, RM; Tsai, W-Y; Lee, P-H; Lee, C-S; Ahsan, H; Zhang, Y-J; Chen, C-J; Santella, RM. (2002). Polycyclic aromatic hydrocarbon-DNA adducts in liver tissues of hepatocellular carcinoma patients and controls. *Int J Cancer* 99:14-21

Chepelev, NL; Moffat, ID; Labib, S; Bourdon-Lacombe, J; Kuo, B; Buick, JK; Lemieux, F; Malik, AI; Halappanavar, S; Williams, A; Yauk, CL. (2015). Integrating toxicogenomics into human health risk assessment: Lessons learned from the benzo[a]pyrene case study. *Crit Rev Toxicol* 45(1):44-52.

Davila, D; Romero, D; Burchiel S. (1996). Human T cells are highly sensitive to suppression of mitogenesis by polycyclic aromatic hydrocarbons and this effect is differentially reversed by alphanaphthoflavone . *Toxicol Appl Pharmacol* 139: 333 – 41.

Dessinenko, MF et al. (1996) Mapping of BPDE DNA adducts in the p53 gene of NHBE cells. *Science* 274:430-432

Duarte-Salles, T; Mendez, MA; Pessoa, V; Guxens, M; Aguilera, I; Kogevinas, M; Sunyer J. (2010). Smoking during pregnancy is associated with higher dietary intake of polycyclic aromatic hydrocarbons and poor diet quality. *Public Health Nutrition* 13, 2034-2043

Duarte-Salles, T; Mendez, MA; Morales, E; Bustamante, M; Rodríguez-Vicente, A; Kogevinas, M; Sunyer J. (2012). Dietary benzo(a)pyrene and fetal growth: effect modification by vitamin C intake and glutathione S-transferase P1 polymorphism. *Environment International* 45, 1-8

Duarte-Salles, T; Mendez, MA; Meltzer, HM; Alexander, J; Haugen, M. (2013). Dietary benzo(a)pyrene intake during pregnancy and birth weight: associations modified by vitamin C intakes in the

Norwegian Mother and Child Cohort Study (MoBa). *Environment international 60C*, 217-223. 2013

Einaudi, L; Courbiere, B; Tassistro, V; Prevot, C; Sari-Minodier, I; Orsiere, T; Perrin, J. (2014). In vivo exposure to benzo(a) pyrene induces significant DNA damage in mouse oocytes and cumulus cells. *Human Reproduction*. 29:548-554.

Gan et al. 2012. Effects of benzo(a)pyrene on the contractile function of the thoracic aorta of Sprague-Dawley rats. *Biomed Environ Sci* 25(5):549-56.

Gibbs, GW; Sevigny M (2007a) Mortality and cancer experience of Quebec aluminum reduction plant workers, part 4: cancer incidence. *J Occup Environ Med* 49:1351–1366.

Gibbs, GW; Sevigny, M. (2007b) Mortality and cancer experience of Quebec aluminum reduction plant workers. Part 3: monitoring the mortality of workers first employed after January 1, 1950. *J Occup Environ Med* 49:1269–1287.

Gibbs, GW; Labrèche, F. (2014) Cancer risks in aluminum reduction plant workers: a review. *JOEM*, 56: S40-S48

Health Canada (2015) Draft "Benzo[a]pyrene in Drinking Water"at: http://www.hc-sc.gc.ca/ewh-semt/consult/_2015/bap/draft-ebauche-eng.php

Jayasundara et al. 2015. AHR2-Mediated Transcriptomic Responses Underlying the Synergistic Cardiac Developmental Toxicity of PAHs. *Tox Sci* 143(2):469-81.

Jeng, HA; Pan, CH; Diawara, N; Chang-Chien, GP; Lin, WY; Huang, CT; et al. (2011) Polycyclic aromatic hydrocarbon – induced oxidative stress and lipid peroxidation in relation to immunological alteration. *Occup Environ Med* 68:653 – 8.

Jules, GE; Pratap, S; Ramesh, A; Hood, DB. (2012) In utero exposure to benzo(a)pyrene predisposes offspring to cardiovascular dysfunction in later-life. *Toxicology*. 295(1-3): 56–67.

Liang, et al. (2014). Adverse effect of sub-chronic exposure to benzo(a)pyrene and protective effect of butylated hydroxyanisole on learning and memory ability in male Sprague-Dawley rat. *J Toxicol Sci* 39(5):739-48.

Kerley-Hamilton, JS; Trask, HW; Ridley, CJ; Dufour, E; Lesseur, C; Ringelberg, CS; Moodie, KL; Shipman, SL; Korc, M; Gui, J; Shworak, NW; Tomlinson, CR. (2012). Inherent and benzo[a]pyrene-induced differential aryl hydrocarbon receptor signaling greatly affects life span, atherosclerosis, cardiac gene expression, and body and heart growth in mice. *Toxicological Sciences* 126(2), 391–404.

Kissel JC. (2011). The mismeasure of dermal absorption. *J Expos Sci Environ Epid*. 21(3):302-9.

Knaapen, AM; Curfs, DM; Pachen, DM; Gottschalk, RW; de Winther, MP; Daemen, MJ; Van Schooten FJ. (2007). The environmental carcinogen benzo[a]pyrene induces expression of monocyte-chemoattractant protein-1 in vascular tissue: a possible role in atherogenesis. *Mutat Res* 621:31 – 41 .

Kummer, V; Maskova, J; Zraly, Z; Faldyna, M. (2013). Ovarian disorders in immature rats after postnatal exposure to environmental polycyclic aromatic hydrocarbons. *Journal of Applied Toxicology*. 33:90-99.

Kurihara-Bergstrom, T., Flynn, G.L., Higuchi, W.I. (1986). Physicochemical Study of Percutaneous Absorption Enhancement by Dimethyl Sulfoxide: Kinetic and Thermodynamic Determinants of Dimethyl Sulfoxide Mediated Mass Transfer of Alkanols. *J Pharm Sci* 75:479-486.

Kyrtopoulos, SA. (2006). Biomarkers in environmental carcinogenesis research: striving for a new momentum. *Tox Lett* 162:3-15.

Maciel, ES; Biasibetti, R; Costa, AP; Lunardi, P; Schunck, RV; Becker, GC; Arbo, MD; Dallegrave, E; Goncalves, CA; Saldiva, PH; Garcia, SC; Leal, RB; Leal, MB. (2014). Subchronic oral administration of Benzo[a]pyrene impairs motor and cognitive behavior and modulates S100B levels and MAPKs in rats. *Neurochem Res* 39:731-740.

Manchester, DK; Weston, A; Choi, J-S; Trivers, GE; Fennessey, PV; Quintana, E; Farmer, PB; Mann, DL; and Harris, CC. (1988) Detection of benzo[a]pyrene diol-epoxide-DNA adducts in human placenta. *Proc. Natl. Acad. Sci. USA.*, 85: 9243-9247.

Miller, BG; Doust, E; Cherrie, JW; Hurley, JF. (2013) Lung cancer mortality and exposure to polycyclic aromatic hydrocarbons in British coke oven workers. *BMC Public Health* 13:962.

Moffat, et al. (2015). Review Article. Comparison of toxicogenomics and traditional approaches to inform mode of action and points of departure in human health risk assessment of benzo[a]pyrene in drinking water. *Crit Rev Toxicol* 45(1):1-43.

Moorthy, B; Miller, KP; Jiang, W; Williams, ES; Kondraganti, SR; Ramos, KS. (2003) Role of cytochrome P4501B1 in benzo[a]pyrene bioactivation to DNA-binding metabolites in mouse vascular smooth muscle cells: evidence from 32P-postlabeling for formation of 3-hydroxybenzo[a]pyrene and benzo[a]pyrene-3,6-quinone as major proximate genotoxic intermediates. *J Pharmacol Exp Ther* 305(1):394-401.

Moorthy, B; Chu C; Carlin, DJ. (2015). Contemporary Review. Polycyclic Aromatic Hydrocarbons: From Metabolism to Lung Cancer. *Tox Sci* 145(1):5-15.

N ' Diaye, M; Le Ferrec, E; Kronenberg, F; Dieplinger, H; Le Vee, M; Fardel, O. (2009). TNF α - and NF- κ B-dependent induction of the chemokine CCL1 in human macrophages exposed to the atherogenic lipoprotein(a). *Life Sci* 84:451 – 7 .

Nanez, A; Alejandro, NF; Falahatpisheh, MH; Roths, JB; Ramos, KS. (2005). Disruption of cell-cell and cell-matrix interactions in hydrocarbon nephropathy. *American Journal of Physiology-Renal* 289(6):F1291-F1303.

Nanez, A; Ramos, IN; Ramos, KS. (2011). A mutant allele of AHR protects the embryonic kidney from hydrocarbon-induced deficits in fetal programming. *Environmental Health Perspectives* 119:1745-1753.

Oesterling, E; Toborek, M; Hennig, B. (2008). Benzo[a]pyrene induces intercellular adhesion molecule-1 through a caveolae and aryl hydrocarbon receptor mediated pathway. *Toxicol Appl Pharmacol* 232:309 – 16.

Olsen, A-K; Andreassen, Å; Singh, R; Wiger, R; Duale, N; Farmer, PB; Brunborg, G. (2010). Environmental exposure of the mouse germ line: DNA adducts in spermatozoa and formation of de novo mutations during spermatogenesis. *PLoS ONE* 5:e11349.

Parrish, AR; Alejandro, NF; Bral, CM; Kerzee, JK; Bowes, RC III; Ramos, KS. (2002). Characterization of glomerular cell phenotypes following repeated cycles of benzo(a)pyrene injury in vitro. *Biochemical Pharmacology* 64(1):31-39.

Patri, M; Singh, A; Mallick, BN. (2013) Protective role of noradrenaline in benzo[a]pyrene-induced learning impairment in developing rat. *J Neurosci Res* 91:1450-1462.

Perera, FP; Chang, HW; Tang, D; Roen, EL; Herbstman, J; Margolis, A; Huang, TJ; Miller, RL; Wang, S; Rauh, V. (2014) Early-life exposure to polycyclic aromatic hydrocarbons and ADHD behavior problems. *PLoS ONE* 9:e111670.

Reiners, JJ; et al. (1984) Dose-response for BaP skin carcinogenesis in two different mouse strains. *Carcinogenesis* 3:301-307

Roelofzen, JH; Aben, KK; Van de Kerkhof, PC; Van der Valk, PG; Kiemeney, LA. (2015) Dermatological exposure to coal tar and bladder cancer risk: a case-control study. *Urol Oncol* 33(1):20.e19-22.

Romundstad, P; Andersen, A; Haldorsen, T. (2000). Cancer incidence among workers in six Norwegian aluminum plants. *Scand J Work Environ Health* 26:461-469.

Ronneberg, A; Haldorsen, T; Romundstad, P; Andersen, A. (1999). Occupational exposure and cancer incidence among workers from an aluminum smelter in western Norway. *Scand J Work Environ Health* 25:207-214.

Rota, M; Bosetti, C; Boccia, S; Boffetta, P; La Vecchia, C. (2014). Occupational exposures to polycyclic aromatic hydrocarbons and respiratory and urinary tract cancers: an updated systematic review and a meta-analysis to 2014. *Arch Toxicol* 88(8):1479-90.

Roy, TA and Singh, R. (2011). Effect of soil loading and soil sequestration on dermal bioavailability of polynuclear aromatic hydrocarbons. *Bull Environ Contam Toxicol*. 67(3):324-331.

Sadeu, JC; Foster, WG. (2011). Effect of in vitro exposure to benzo a pyrene, a component of cigarette smoke, on folliculogenesis, steroidogenesis and oocyte nuclear maturation. *Reproductive Toxicology* 31:402-408.

Sadeu, JC; Foster WG. (2013). The cigarette smoke constituent benzo a pyrene disrupts metabolic enzyme, and apoptosis pathway member gene expression in ovarian follicles. *Reproductive Toxicology* 40:52-59.

Sarto, F; Zordan, M; Tomanin, R; et al. (1989) Chromosomal alterations in peripheral blood lymphocytes, urinary mutagenicity and excretion of polycyclic aromatic hydrocarbons in six psoriatic patients undergoing coal tar therapy. *Carcinogenesis* 10:329-334.

Saperstein, MD; Wheeler, LA. (1979) Mutagenicity of coal tar preparations used in the treatment of psoriasis. *Toxicol Lett* 3:325-329.

Shendrikova; Aleksandrov. (1974). Comparative penetration of polycyclic hydrocarbons through the rat placenta into the fetus. *Bull Exp Biol Med* 77(2): 169–171

Spalt, EW; Kissel, JC; Shirai, JH; Bunge, AL. (2009). Dermal absorption of environmental contaminants from soil and sediment: A critical review. *J Expos Sci Environ Epid* 19:119-148.

Spinelli, JJ; Band, PR; Svirchev, LM; Gallagher, RP. (1991). Mortality and cancer incidence in aluminum reduction plant workers. *J Occup Med* 33:1150-1155.

Stribinskis, V; Ramos, KS. (2006). Activation of human LINE-1 retrotransposition by benzo(a)pyrene. *Cancer Research* 66(5):2616-2620.

Stroo, HE; Roy, TA; Liban, CB; Kreitinger, JP. (2005) Dermal Bioavailability of Benzo[a]Pyrene on Lampblack: Implications for Risk Assessment. *Environ Toxicol Chem* 24:1568-1572.

Swaen, GMH; Slangen, JJM; Volovics, A; et al. (1991). Mortality of coke plant workers in the Netherlands. *Br J Ind Med* 48:130-135.

Teneng, I.; Montoya-Durango, D.E.; Quertermous, J.; Lacy, M.E.; Ramos, K.S. Reactivation of L1 retrotransposon by benzo(a)pyrene involves complex genetic and epigenetic regulation. *Epigenetics* 6, 355-367, 2011. PMID 21150308.

Thakur, VS; Liang, YW; Lingappan, K; Jiang, W; Wang, L; Barrios, R; Zhou, G; Guntupalli, B; Shivanna, B; Maturu, P; Welty, SE; Moorthy, B; Couroucli, XI. (2014). Increased susceptibility to hyperoxic lung injury and alveolar simplification in newborn rats by prenatal administration of benzo[a]pyrene. *Tox Lett* 230: 322-332.

Uno et al. (2014). Protective role of cytochrome P450 1A1 (CYP1A1) against benzo[a]pyrene-induced toxicity in mouse aorta. *Toxicology* 316:34-42.

Valentovic, MA; Alejandro, N; Brown, PI; Ramos, KS. (2006). Streptozotocin (STZ) diabetes enhances benzo(a)pyrene-induced renal injury in Sprague Dawley rats. *Toxicology Letters* 164(3):214-220.

Verhofstad, N; La Pennings, J; van Oostrom, CTM; van Benthem, J; van Schooten, FJ; van Steeg, H; Godschalk, RWL. (2010a). Benzo(a)pyrene induces similar gene expression changes in testis of DNA repair proficient and deficient mice. *BMC Genomics*. 11:333.

Verhofstad, N; van Oostrom, CTM; van Benthem, J; van Schooten, FJ; van Steeg, H; Godschalk RWL. (2010b). DNA Adduct Kinetics in Reproductive Tissues of DNA Repair Proficient and Deficient Male Mice After Oral Exposure to Benzo(a)pyrene. *Environmental and Molecular Mutagenesis* 51:123-129.

Verhofstad, N; van Oostrom, CTM; Zwart, E; Maas, LM; van Benthem, J; van Schooten, FJ; van Steeg, H; Godschalk, RWL. (2011). Evaluation of Benzo(a)pyrene-Induced Gene Mutations in Male Germ Cells. *Toxicological Sciences* 119:218-223.

Wester, P; Muller, J; Slob, W; Mohn, G; Dortant, P; Kroese, E. (2012). Carcinogenic activity of benzo[a]pyrene in a 2 year oral study in Wistar rats. *Food Chem Toxicol* 50:927– 35

Xu, G; McMahan, CA; Walter, CA. (2014). Early-Life exposure to benzo[a]pyrene increases mutant frequency in spermatogenic cells in adulthood. *PLoS ONE* 9:e87439.

Xu, Z; Brown, LM; Pan, GW; et al. (1996). Cancer risks among iron and steel workers in Anshan, China, Part II: Case-control studies of lung and stomach cancer. *Am J Ind Med* 30:7-15.

Yang, JJ; Roy, TA; Krueger, AJ; Neil, W; Mackerer, CR. (1989) Percutaneous Absorption of Benzo(a)Pyrene from Soils with and without Petroleum Crude Contamination. In *Petroleum Contaminated Soils*; Calabrese, E. J., Kostecki, P. T., Eds.; Lewis Publishers: Chelsea, MI, Vol. 2, pp 399-407.

Yang. H; Zhou, L; Wang, Z; Roberts, LJ II; Lin, X; Zhao, Y; Guo, Z . (2009). Overexpression of antioxidant enzymes in ApoE-defi cient mice suppresses benzo(a)pyrene-accelerated atherosclerosis. *Atherosclerosis* 207:51 – 8.

Zaccaria; McClure. (2013). Using immunotoxicity information to improve cancer risk assessment for polycyclic aromatic hydrocarbon mixtures. *Int J Toxicol* 32(4):236-50.

Zanardi, F; Salvarani, R; Cooke, RM; Pirastu, R; Baccini, M; Christiani, D; Curti, S; Risi, A; Barbieri, A; Barbieri, G; Mattioli, S; Violante, FS. (2013) Carcinoma of the pharynx and tonsils in an occupational cohort of asphalt workers. *Epidemiology* 24:100–103.

Zhao; et al. (2014). Exposure of mice to benzo(a)pyrene impairs endometrial receptivity and reduces the number of implantation sites during early pregnancy. *Food Chem Toxicol* 69:244-251.

Additional Peer-reviewed studies contained in HERO

The SAB recommends that EPA consider the following peer-reviewed studies contained in HERO but that are not cited within the BaP document:

Gunter, MJ; Divi, RL; Kulldorff, M; Vermeulen, R; Haverkos, KJ; Kuo, MM; Strickland, P; Poirier, MC; Rothman, N; Sinha, R. (2007). Leukocyte polycyclic aromatic hydrocarbon-DNA adduct formation and colorectal adenoma. *Carcinogenesis* 28(7):1426-1429.

Poirier, M.C. (2012). Chemical-induced DNA damage and human cancer risk. *Discovery Medicine* 14(77):283-288.

Rothman, N; Correa-Villasenor, A; Ford, DP; Poirier, MC; Haas, R; Hansen, JA; O'Toole, T; Strickland, PT. (1993). Contribution of occupation and diet to white blood cell polycyclic aromatic hydrocarbon-DNA adducts in wildland firefighters. *Cancer Epidemiology Biomarkers and Prevention* 2:341-347.

Sinha, R; Kulldorff, M; Gunter, MJ; Strickland, P; Rothman, N. (2005). Dietary benzo[a]pyrene intake and risk of colorectal adenoma. *Cancer Epidemiology Biomarkers and Prevention* 14(8):2030-2034.

APPENDIX C: SUGGESTIONS ON THE FORMAT FOR EPA's CHARGE QUESTIONS

The format for EPA's charge questions for the SAB review of the IRIS Toxicological Review of Benzo[a]pyrene is different than that for previous IRIS assessments. The CAAC-BaP panel would like to offer the following suggestions based on the experience during panel review of this assessment:

1) Charge questions on hazard identifications should not consist of a separate charge question for all critical endpoints. This is because the first step in the development of toxicity values involves the selection of critical studies and endpoints. Thus, the discussion on critical effects became redundant during the review meeting.

2) Charge questions on the development of RfD, RfC, oral slope factor, IUR, and dermal slope factor actually involve many subparts that should be reviewed by panel members with very different expertise. Separate charge questions should be provided for each subpart (e.g., selection of critical studies and effect, determination of the point of departure, derivation of the toxicity value, uncertainty analysis) arranged in a logical sequence. This will make the assignment of lead discussants for each subpart of the charge question clearer.

3) For the charge question on EPA's response to public comments, the major science issues pointed out by public commenters should be included in the relevant charge questions (or subparts of the charge question). The SAB can then comment on whether EPA's approach is scientifically supported. The SAB should not be asked if EPA has adequately addressed all public comments.